Collection Forum

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SURVEY OF GASEOUS POLLUTANT CONCENTRATION DISTRIBUTIONS IN MINERAL COLLECTIONS

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Abstract.—The concentrations of four types of gaseous pollutants were semiquantitatively determined in samplings of cabinets from three mineral collections. The pollutants measured included: acidic vapours (thought to be primarily carboxylic acid vapours), mercury vapour, sulfur dioxide, and silver-tarnishing gasses (thought to be primarily hydrogen sulfide). These pollutants were monitored using simple and inexpensive monitors and/or dosimeters. Some of the monitors and dosimeters were commercially available, whereas others were specially fabricated and calibrated. The three investigated collections provided examples of three material combinations: metal drawers in metal cabinets, wooden drawers in metal cabinets and wooden drawers in wooden cabinets. Interpretation of pollutant concentrations as functions of position within the crystal-chemical classification system used to organise collection storage generally showed anticipated patterns. Several exceptions to these patterns, which have significant implications for collection care, were also noted.

Research about museum pollutants has largely dealt with the environment external to the museum building but, more recently, with pollutants generated by the materials used in the construction of museum furniture, fittings, and displays. Summaries of pollutant research relevant to museums are provided by Padfield et al. (1982), Thomson (1986), Brimblecoombe (1989), Haiad et al. (1990), and Grzywacz (1995). Recent research has concentrated on the identification of efflorescent growths on museum objects (for example Tennent and Baird 1985, 1992, Tennent et al. 1993), however, relatively little research has been carried out on pollutants generated by the geological specimens themselves. In spite of this, any geological collection worker will be familiar with pyrite decay and its characteristically unpleasant sulfurous smell. In addition, some specimens are volatile and their vapours could constitute internal pollutants (Waller 1992).

This study was designed to obtain information on the effects of the cabinet construction materials and the mineral species present on the types and levels of gaseous pollutants present. The work described here had three main goals: (1) to adapt or develop low-cost, semiquantitative pollutant indicators for use in cabinets, (2) to evaluate the effect of different types of storage cabinets on pollutant levels, and (3) to evaluate the distribution of pollutant concentration levels within the crystal-chemical ordering of systematic mineral collections.

Three collections were chosen to provide examples of three different storage material combinations: metal drawers in metal cabinets, wooden drawers in metal cabinets, and wooden drawers in wooden cabinets. The optimum size of collection for this project was considered to be 100–200 cabinets. This number of cabinets would allow most of the cabinets to be tested and would permit reasonable resolution of pollutant level variations as a function of the mineral species present. The collections used for the field testing were those of the Canadian Museum of

Nature (CMN) in Ottawa, the Geological Survey of Canada (GSC) in Ottawa, and the Royal Ontario Museum (ROM) in Toronto.

DESCRIPTION OF THE COLLECTIONS TESTED

All of the collections were arranged systematically according to common crystal-chemical classification schemes, starting with native elements, then sulfides, and ending with silicate minerals. All collections used acidic, lignin-containing cardboard specimen trays as containers for most specimens. At the Canadian Museum of Nature, some of these trays had an over-wrap of alkali-buffered paper. The Geological Survey of Canada and the Canadian Museum of Nature utilise a classification based on Dana's system of mineralogy (Palache et al. 1944, 1951) for the nonsilicate minerals. The Royal Ontario Museum uses the revised Dana numbers found in Ferraiolo (1982) for the nonsilicate minerals. All three collections use a crystal-structural classification for the silicate minerals starting with orthosilicates and ending with tectosilicates. The Geological Survey of Canada and the Canadian Museum of Nature use Strunz (1970) as the guide to classification. The Royal Ontario Museum uses Dana's Textbook of Mineralogy (Ford 1932) in conjunction with Deer et al. (1966) and specialised recent publications such as Hawthorne (1983) and Bailey (1988) as guides.

Canadian Museum of Nature Collection

The Canadian Museum of Nature collection is housed in standard geological, all-metal cabinets measuring $81 \times 74 \times 69$ cm. The majority of the cabinets are finished with baked alkyd enamel; a few are finished with a baked acrylic coating. Cabinets were all at least 2 yr old at the time of the study. Sampling included all of the cabinets containing nonsilicate mineral species and every second cabinet containing silicate minerals. A total of 148 cabinets were sampled out of the population of 192 cabinets used to store the collection.

Geological Survey of Canada Collection

The Geological Survey of Canada collection is housed in metal cabinets measuring $192 \times 64 \times 66$ cm, finished with what was thought to be a baked alkyd enamel and containing unfinished softwood drawers. All 55 of these double-height cabinets were sampled. These cabinets were thought to be in excess of 20 yr old.

Royal Ontario Museum Collection

The Royal Ontario Museum collection is housed in cabinets, $82 \times 50 \times 58$ cm, made either of birch plywood, or of a close-grained hardwood. The hardwood cabinets are described by the staff as being made of cherry wood. All cabinets were sampled from native elements to oxides, except in oxides where three or more cabinets contained the same species. In those situations, only the first and last cabinets containing the species were sampled. From halides, to carbonates, to other oxysalts, inclusive, every fifth cabinet was sampled. Only five of the 134 silicate cabinets were sampled because of time constraints. A total of 171 cabinets were sampled out of a population of 398 cabinets.

POLLUTANTS CONSIDERED

Because of their importance in deterioration reactions affecting museum collections, four kinds of gaseous pollutants were studied. These included carboxylic acids, sulfur dioxide, mercury vapour, and reduced sulfur gasses.

Carboxylic Acids

Acetic and other carboxylic acids are known to be emitted by materials such as wood, coatings, adhesives, sealants, etc. used to construct storage cabinets and display cases (Miles 1986, Padfield et al. 1982, Tétreault 1992, Tétreault and Stamatopoulou 1997). Acetic and formic acids, emitted by wood, have been identified as the cause of acetate and formate salt formation on calcareous specimens commonly known as Byne's disease (Agnew 1981, Grzywacz and Tennent 1994, Nicholls 1934, Tennent and Baird 1985). Acetic acid will corrode metals, particularly lead, but also other metals (Blackshaw and Daniels 1979, Green 1989, Padfield et al. 1982, Tennent et al. 1993, Tétreault et al. 1998). Carboxylic acids are also thought to be responsible for dulling the surfaces of certain borate minerals (Erd pers. comm. 1991) and, presumably, other minerals that are salts of weak acids.

Sulfur Dioxide

Sulfur dioxide is a major product of pyrite oxidation at intermediate to low relative humidity levels (Waller 1990). It is formed according to the reaction:

$$FeS_2 + H_2O + 3O_2 \Rightarrow FeSO_4 \cdot H_2O + SO_2$$
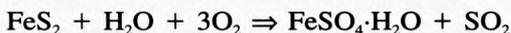
(at 25°C and low to moderate relative humidity). Sulfur dioxide dissolves to form moderately acidic sulfurous acid, which in turn is readily oxidised to form very strong sulfuric acid solutions. It is known to affect a wide variety of materials in museums (Thomson 1986). At higher levels of relative humidity, much of the sulfur dioxide is oxidised *in situ* to form sulfuric acid. Migrating sulfuric acid will react with monosulfide minerals to produce hydrogen sulfide as described below and will char paper labels, trays, and even wooden drawers.

Mercury

Mercury has a low but significant vapour pressure, 0.0018 mm of Hg at 25°C (Dean 1978). Mercury vapour is emitted by specimens containing native mercury and possibly by other mercury-containing minerals. In addition to being a potential health hazard, and depleting the mercury specimens themselves, the mercury emitted can form solid solutions with other native metal mineral species, altering their chemical composition.

Reduced Sulfur Gases

Numerous gases containing reduced sulfur are known to exist as pollutants (Graedel 1984). The most significant of these in terms of sulfidation reactions are thought to be carbonyl sulfide (OCS) and hydrogen sulfide. (H_2S) (Brimble-coombe et al. 1992, Franey et al. 1985, Soto et al. 1982). Within mineral collections, elemental sulfur vapour may also contribute to sulfidation reactions. Hydrogen sulfide is released when the acid solutions generated during pyrite oxidation react with monosulfide minerals according to reactions such as:

$$FeS + H_2SO_4 \Rightarrow H_2S + FeSO_4$$
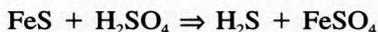
Hydrogen sulfide might also be generated by hydrolysis of simple sulfides by atmospheric moisture. Hydrogen sulfide and other reduced-sulfur gases are known to tarnish silver mineral specimens (see, for example, Pearl 1975).

Other Gaseous Pollutants

Many gasses and vapours known or suspected of being present in mineral collections were not monitored. Both oxygen and carbon dioxide can cause deterioration of specimens (Howie 1984, Waller 1992) but these common atmospheric gasses were not considered in this study. Other pollutant gasses known to be present in mineral collections, but not measured as part of this study, include formaldehyde and radon.

Finally, it is probable that other gasses occur as pollutants in collections. For example, boric acid and the arsenic and selenium analogues of hydrogen sulfide might be expected to occur, in at least trace amounts, in some parts of collections although this has not been documented. This study was not intended to identify exhaustively all pollutants present, but, rather, to determine concentration distributions of selected gaseous pollutants.

METHODS

Five types of test strips were selected for use in the study: metal (copper, silver, and lead) foils, lead acetate papers, palladium chloride papers, pH test strips, and sulfite ion test strips. These were either purchased off-the-shelf or manufactured in-house. The fabrication of purpose-made strips, and the calibration, photography of results, and testing procedures are described below for each of the five types of test strip deployed.

Metal Test Strips

Manufacture.—Metal test strips comprised 5 × 5 mm coupons (pieces) of copper, silver, and lead foils, degreased with acetone and adhered to Mylar® (polyethylene terephthalate) drafting film, with Rhoplex N-560 (Appendix 1), a pressure-sensitive acrylic adhesive. The metals used were 99.999% pure copper and lead and 99.9% pure silver.

Calibration: acetic acid.—A series of concentrations of acetic acid in water were used to make saturated solutions and excess salt mixtures with magnesium nitrate hexahydrate $(Mg(NO_3)_2 \cdot 6H_2O)$. These solutions provided a range of eight acetic acid concentrations (Table 1) at a fixed relative humidity level of 54% (Tétreault et al. 1998). The solutions, contained in small disposable beakers, were placed in screw-topped glass jars with a test strip mounted onto a second inverted beaker. The metal test strips were exposed to these mixtures over a period of 13 wk. This exposure period then dictated the duration of field testing of collections. In this way, a direct comparison of corrosion obtained in collection cabinets to corrosion obtained in calibration exposures was possible. A range of states of corrosion was obtained on the lead foil coupons after 3 mo. The colour graduations obtained varied from untarnished up to 1.32 ppm, pale blue-grey tarnish at 4.13–7.74 ppm, darker blue-grey tarnish between 12.4 and 20.6 ppm, a patchy white efflorescence at 25.8–41.2 ppm, and a solid coating of white efflorescence at 51.6 ppm.

Table 1. Concentrations of acetic acid in the gas and $Mg(NO_3)_2 \cdot 6H_2O$ saturated solution phases. Gaseous phase concentrations calculated from Clarke and Longhurst (1961).

Concentration of acetic acid in gaseous phase/ppm (volume/volume)	% of acetic acid in liquid phase (volume/volume)
0.00	0.00
1.32	0.025
4.13	0.077
7.74	0.14
12.4	0.23
20.7	0.38
25.8	0.48
41.2	0.77
51.6	0.96

Calibration: hydrogen sulfide.—Because of technical difficulties, the calibration of metal test strips for hydrogen sulfide could not be done at the low concentrations and long exposure times encountered during field testing. Instead, a series of exposures at fixed concentration and varying time was used to establish a scale related to concentration-time dosage. For example, a strip exposed to hydrogen sulfide at 27 ppb for 9 days was said to have received a dose of 27 ppb × 9 days or 243 ppb-days hydrogen sulfide.

Atmospheres of fixed hydrogen sulfide concentration and 50% relative humidity were generated using a permeation wafer device (Appendix 1) (Andrew et al. 1993). Calibration exposures were made for periods between 1 and 23 days at 27 ppb hydrogen sulfide and 50% relative humidity. Test strips were inserted approximately twice a week to provide a nine-step series of concentration-time exposures. Iridescent blue tarnish formed very rapidly on the copper; brown, and then black, tarnish formed much more slowly on the silver. The lead showed a variety of interference colours between a dull blue, through shiny brown to pronounced shiny blue. Test strips were photographed together with a colour scale. The exposed test strips were encapsulated in Mylar® for use in field-testing. Metal test strips were also exposed to a combination of 25 ppb hydrogen sulfide and 0.7 ppm sulfur dioxide at 50% relative humidity to determine the combined effect of these pollutants. This level of sulfur dioxide roughly doubled the extent of sulfidation for concentration time dosages of up to 300 ppb-days hydrogen sulfide. See Table 2.

Lead Acetate Papers

Manufacture.—A commercial lead acetate test paper was employed (Appendix 1) and a buffered lead acetate paper was made by soaking the commercial lead acetate paper in a 1 M solution of sodium carbonate. The paper was buffered to retard acidification that would otherwise result in reduced sensitivity of the lead acetate to hydrogen sulfide. Both types of lead acetate paper were dampened before use with an 80% w/w glycerol/water solution.

Calibration.—The lead acetate and buffered lead acetate test strips were exposed to the same concentration-time dosage regime as the metal test strips, described above. The unbuffered lead acetate papers showed a variety of colours

Table 2. Mapping of responses of copper foil test strips to 27 ppb of H₂S at 50% relative humidity against responses to 25 ppb of H₂S and 0.7 ppm of SO₂ at 50% relative humidity.

0 ppm sulfur dioxide		0.7 ppm sulfur dioxide	
Strip no.	ppb H₂S-days	Strip no.	ppb H₂S-days
19	0		
18	30		
17	55	8	25
		7	50
16	135		
15	245	6	125
14	350		
13	435	5	300
12	500	3	550
11	620		

from yellow, through light reddish brown, dark brown, to black and silvery grey. The buffered lead acetate papers ranged from beige through brown to black.

Palladium Chloride Paper

Manufacture.—A test paper for mercury vapour, based on the spot test described by Feigl and Anger (1972), was made by dipping filter paper into a slightly acidified solution of palladium chloride, PdCl₂ (Appendix 1).

Calibration: mixed pollutants.—The palladium chloride paper was exposed to the same concentration-time dosage regime as the metal test strips, described above. Exposed to pure hydrogen sulfide, the test papers became a slightly mottled grey-brown; exposed to mixed hydrogen sulfide and sulfur dioxide, the papers developed a yellow to yellow-brown colour. Neither of these discolourations resembled the silver-grey reaction to mercury.

Calibration: mercury.—Test strips were taped onto the lid of a screw-top jar containing an excess of liquid mercury at 25°C and 50% relative humidity. At this temperature, the vapour pressure of mercury is 0.0018 mm of Hg (Dean 1978). The surface area of mercury in the jar was much greater than the surface area of the test strips (50 : 0.3 cm²). Consequently, the air in the jar was assumed

Table 3. Concentration time dosages of mercury employed for calibration of the palladium chloride test paper. For the longest exposure, the values in parentheses reflect experimental conditions, whereas the values in italics were assigned based on the colourimetric evaluation described in the text.

Strip no.	Time (minutes)	Exposure (ppm-days Hg)	Intermediate values assigned (ppm-days Hg)
0	0	0	
			0.0416
7	50	0.0833	
			0.1833
6	170	0.2833	
			0.3833
5	290	0.4833	
			0.54 (1.444)
4	1,440	*0.59* (2.43)	
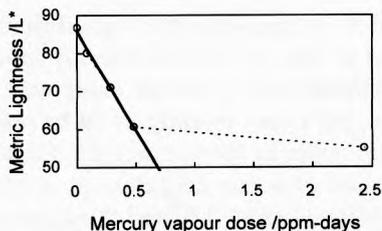
Figure 1.   Metric brightness (L*) versus concentration-time dosage of mercury in ppm-days for the palladium chloride test strips during calibration. Solid line shows the least squares fit for the first four data points.

to be saturated with mercury vapour and the concentration of mercury in the jar was calculated to be 0.0018/760 or 2.37 ppm. As with the metal strips exposed to hydrogen sulfide, a concentration-time calculation was employed (Table 3).

The test strip given the highest concentration-time dose (2.43 ppm-days) appeared to be out of line with the others. This was thought to result from the test strips becoming saturated with respect to colour at a concentration-time dose lower than the highest exposure used for calibration. Measurements of metric lightness (L*) taken with a Minolta model CR-241 chromameter were used to relate the appearance of the calibration test strips to the concentration-time exposure. Figure 1 shows this relationship together with a line showing the least squares fit of the first four data points. The test strips exposed to <0.5 ppm-days of mercury show a near linear relationship between metric lightness and exposure dose. In contrast, the strip exposed to 2.43 ppm-days of mercury has a much higher metric lightness than would be expected by considering the other test strips. The linear regression fit for the first four data points was extrapolated to L* = 55 to determine that a concentration-time dose of 0.59 ppm-days could be sufficient to cause a metric lightness of 55 in the test strip. Concentration-time dosages recorded in cabinets were subsequently adjusted to reflect this. Any cabinet for which a mercury concentration equivalent to 0.59 ppm-days was recorded may, of course, have had a higher level.

During field testing, it became obvious that the reaction of the palladium chloride test paper to the mixtures of the various pollutants was complex. After 72 hr in the field tests, nearly all the palladium chloride strips had developed a light grey spotting, although those in cabinets known to contain mercury minerals had become a uniform silver grey. Test strips left in the room air for 3 mo also developed a light grey spotting.

The sensitivity of the palladium chloride test strips declined over a 1-yr period following preparation, despite being stored in a sealed desiccator over dry Drierite®. The exact nature and cause of this deterioration are not understood. In practical terms, the consequence of this loss of sensitivity is that test strips must be calibrated at the time of use. Test strips were calibrated and used in this project about 6 mo after preparation.

pH Test Strip

A nonbleeding pH indicator strip for the range of 0–6 made up of three narrow-range indicator papers was used (Appendix 1). It was dampened with an 80%

w/w glycerol/water solution to measure the equilibrium pH (EqpH) of the air. Equilibrium pH is defined as the pH obtained in an aqueous solution in equilibrium with the air. The manufacturer's colour chart was used to interpret colour responses as pH units. The pH paper reading at 24 hr provided an estimate of the concentration of carboxylic acids in the cabinet air. These readings can be related to an equivalent concentration of acetic acid through a calibration curve (Tétreault 1992). Equilibrium pH readings below 3.6, and those taken after 3 mo, are thought to be influenced by the effects of other acids, such as sulfuric acid formed by the oxidation of sulfur dioxide.

Sulfite Ion Test Paper

Quantofix® sulfite ion test paper (Appendix 1), dampened with an 80% w/w glycerol/water solution, was used to detect sulfur dioxide. Sulfur dioxide will dissolve and dissociate in the glycerol solution giving a positive reaction for sulfite ions. The manufacturer's colour chart (calibrated for use in aqueous solutions) was used to interpret colour responses. Calibration of colour responses for gas phase exposures was not available. However, during one experiment, a test strip exposed to a concentration of 6.8 ppm of sulfur dioxide yielded a maximum colour chart response of 25 ppm of sulfite.

The sulfite detecting paper took time to develop a maximum colour, which then faded over time. This reduction in intensity of colour appeared to be linked to a drop in pH below 3.5. The product literature states that the paper will not work at low pH but does not specify a limit for functionality. Continued reduction in pH is a result of *in situ* oxidation of sulfite to sulfate and the rate of this reaction is known to be relative humidity dependent (Waller and McAllister 1987). Consequently, the time required to achieve a maximum response in the test strips is expected to be relative humidity dependent. A test was run to record the indicated sulfur dioxide concentration, on the sulfite ion test strip, over time inside mineral cabinets. The cabinets selected for testing were those containing disulfide species, such as pyrite and marcasite, at the Canadian Museum of Nature. They were known to contain high levels of sulfur dioxide and be at a relative humidity of approximately 35%. It was found that, within uncertainty of test strip readings, the maximum response to SO_3^{2-} was recorded after 72 hr of exposure (Fig. 2). In the light of this result, it was decided to record test strip responses at 72 hr of exposure in cabinets.

Reading Results from Test Strips

Table 4 is a compilation of the concentration-time dosages received by manufactured test strips during the calibration experiments and the levels indicated by calibration charts for commercially available test strips, together with intermediate levels assigned. Intermediate levels were recorded during the survey when a test strip showed a response that lay between two of the calibration increments. All graphs of pollutant concentration levels included in this paper use these increments.

Commercial test strips.—The sulfite ion paper was compared to the colour chart printed on the product container. Colours below 10 on the scale were difficult to evaluate; the value 5 was assigned for a distinct colour; the value 2 for an indistinct colour. The pH paper was compared to the colours on a composite chart
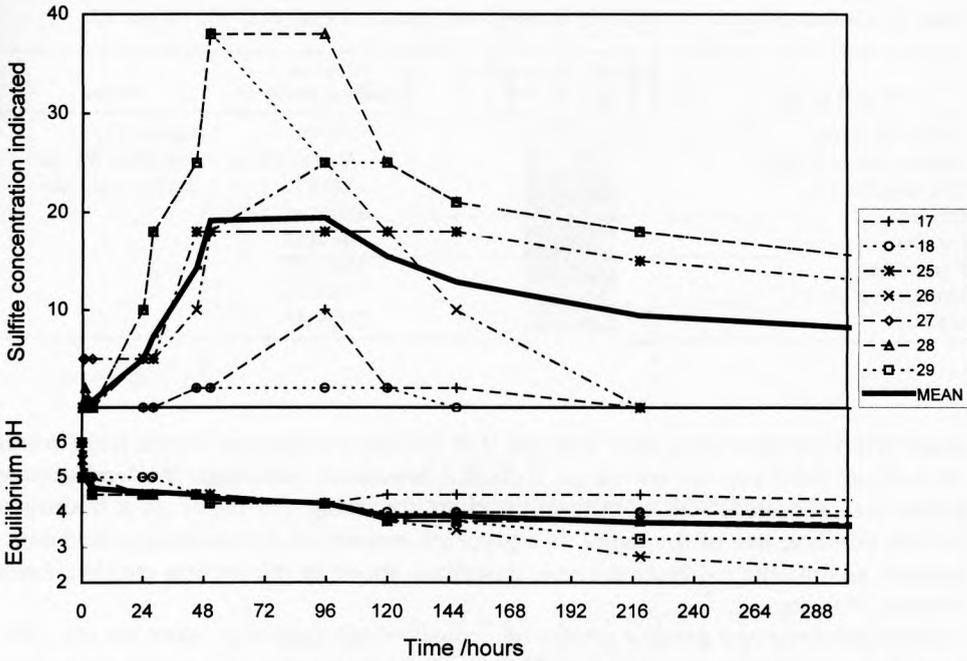
Figure 2.   Sulfite test strip and pH paper responses as a function of time during exposure in selected cabinets in the Canadian Museum of Nature mineral collection. Cabinet numbers in legend refer to cabinet sequence numbers.

Table 4.   Calibration steps and intermediate increments for various pollutants. Intermediate increments are shown in italics.

Acetic acid on lead foil (ppm)	Hydrogen sulfide (ppb-days)	pH	SO₂ on Quantofix (scale arbitrary)	Hg on PdCl (ppm-days)
0	0	3.0	0	0
0.66	*10*	3.25	2	*0.04*
1.32	30	3.5	5	0.08
2.73	*40*	*3.55*	10	*0.18*
4.13	55	3.6	*18*	0.28
5.64	*95*	*3.8*	25	*0.38*
7.14	135	4.0	*38*	0.48
9.8	*190*	*4.25*	50	*0.54*
12.4	245	4.4	*75*	0.59
16.6	*300*	*4.45*	100	
20.7	350	4.5		
23.3	*390*	*4.55*		
25.8	435	4.7		
33.5	*500*	*4.8*		
41.2	565	5.0		
46.4	*595*			
51.6	620			

Table 5. Cabinet contents and sequence numbers and legend for Figures 5, 10, 12, and 15.

Mineral group	Legend for figures 5, 10, 12, 15	Cabinet sequence numbers	Notes
Native elements		1–34	sulfur: 31, 32
Sulfides and sulfosalts		34–76, 88–110	cinnabar: 60–62
Iron disulfides		76–87	pyrite, marcasite
Oxides		110–146	
Halides		146–153	
Carbonates		154–177	
Other oxysalts		178–200	
Silicates		201–245	

made from the chart provided with the 0–6 indicator strips and charts from boxes of each of the three narrow-range indicator segments. Although the lead acetate paper was supplied with a calibration chart for using the paper as a hydrogen sulfide monitor, use of the paper as a glycerol-moistened dosimeter required comparison against the calibration series described above in the section entitled, *Lead Acetate Papers.*

Manufactured test strips.—Although considerable care was taken during photography and colour printing, the photographic images were poor representations of some of the indicators. In particular, interference colours on the metal foil squares were not captured. Therefore a combination of the photographs and of the actual samples, sealed in Mylar® envelopes, were used to record results in collections.

In some cases, the actual test strips could not be used for comparison because they had become altered by one of two pathways. In the case of the lead acetate papers, the colours faded considerably over a few months. With the lead strips and palladium chloride paper, the low dosage and control test strips had continued to react with pollutants that presumably, were being emitted from the strips exposed to higher concentration-time dosages. The control from the palladium chloride paper became grey and the lead strips from the acetic acid test developed a uniform matte grey colour. In these instances, the photographs alone were used to evaluate the test strips in collections.

Correlation of Data Between Collections

Each of the collections studied employed similar, but not identical, crystal-chemical ordering systems. Correlation of data between the three collections was made possible by the application of a modification of the classification system of Ferraiolo (1982). The application of this modified system affected the position within the classification system of less than 1% of all mineral species present. Because each collection had widely differing numbers of cabinets, cabinet sequence numbers were assigned to give a numeric ordering common to all three collections based on the modified and extended Ferraiolo classification system. The cabinet contents for each collection as they appear in the assigned sequence numbers are shown in Table 5. The overall order of the collections was not affected, neither were specimens physically moved.
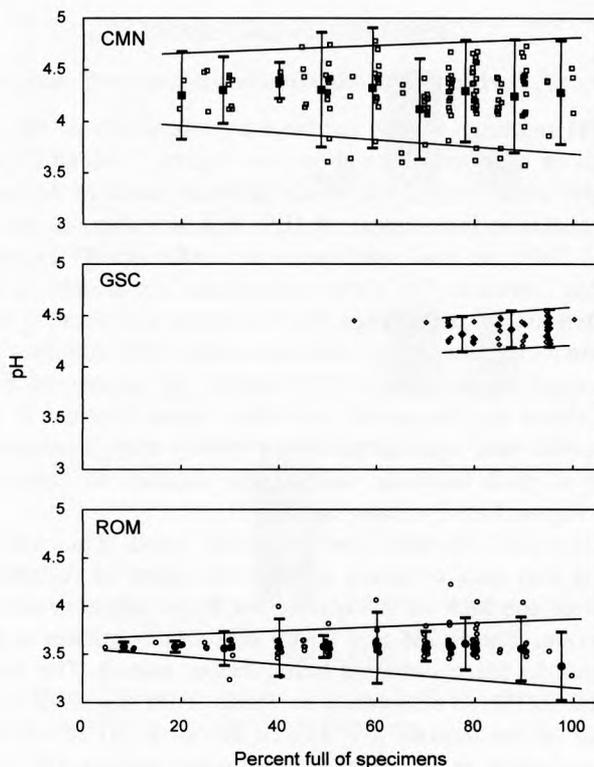
Figure 3. Equilibrium pH readings as a function of quantity of specimens held in cabinets. Error bars above and below mean values reflect ±2 standard deviations. Lines show the linear regression trends of the ±2 standard deviations. Data points have been randomly dispersed around the measured values to better illustrate distribution of measurements. CMN, Canadian Museum of Nature; GSC, Geological Survey of Canada; ROM, Royal Ontario Museum.

Survey Method

The surveys were conducted during 1991, from May to September. Test strips were placed near the centre of the top drawer-front in each cabinet. Readings of the test strips were taken at 24 hr (for pH only), 72 hr, and 3 mo. A series of observations were made of each cabinet. These included: (1) the mineral species present (the start and finish of the series were recorded), (2) the type of cabinet, (3) the number of drawers, (4) an estimate of the fullness of the cabinet (how many specimens were contained over how many like-sized specimens could be contained), (5) the presence (or absence) and type of gasket, (6) the presence of any odour, (7) any damage to the cabinet, and (8) if present, the quantity of silica gel in the cabinet. A photograph was also taken of each cabinet using 35 mm colour slide film.

The relative humidity level (RH) was measured inside several cabinets in each collection. Cabinets at the CMN were a constant 38% RH, at the ROM they ranged from 41–44% RH, the less well scaled cabinets at the GSC ranged from 35–50% RH. The room air in each of the three collection areas was recorded as having an EqpH of 4.5.

RESULTS AND DISCUSSION

Indicators of Carboxylic Acids Concentration—pH and Lead Foil

Equilibrium pH readings within cabinets as a function of the extent to which the cabinet is full of specimens are shown in Figure 3. Mean EqpH, ±2 standard deviations, and the linear regression trends of these standard deviations, calculated for groups of cabinets at increments of 10% full, are also shown. The graphs for CMN and ROM indicate that specimens are influencing the chemistry of the atmosphere within cabinets. For these collections, the trends in the ±2 standard deviations broaden as the percentage full increases and narrow toward the mean for each collection as the percentage full decreases. This indicates that the quantity of specimens present in the cabinet influences the extent of EqpH variations. Deviations both above and below the collection mean increase in increasingly full cabinets. This proves that specimens, and possibly their associated labels, trays, etc., are capable of both reacting with acidic vapours to increase the EqpH or releasing acidic vapours to decrease the EqpH.

The data for the GSC do not show the same trend. This may be, in part, an artefact of having less data covering a narrower range of fullness. It is probably real and a result of the lack of gasketting on these cabinets and consequently a high ventilation rate. The ROM and CMN both show greater scatter in the data, which is attributed to these cabinets being better sealed. The reduced scatter in the data from the ROM as compared to those from the CMN is thought to be primarily a result of the overall low EqpH. Because pH is related to the log of concentration a variation in EqpH of 0.2 pH units around pH = 3.5 may reflect a 10-fold greater acidic vapour concentration than the same variation around pH = 4.5. Nonetheless, the narrowness of the ROM data relative to the CMN data, especially at low levels of percent full, indicates the extent to which the wood of the ROM cabinets controls the EqpH. Attempts to correlate EqpH in cabinets with the type and condition of cabinet gaskets within the CMN and ROM collections provided no useful insights. This was probably the result of both the difficulty in specifying the efficiency of gaskets on the basis of a quick visual inspection and the overwhelming effects of cabinet contents.

Measurements of EqpH at 24 hr were initially thought to provide the best indication of total concentration of carboxylic acids. Figure 4 shows that EqpH readings at 24 hr for each collection were approximately normally distributed. This permits calculation of a confidence interval associated with the mean for each collection. The hypothesis that EqpH at 72 hr would be significantly affected by sulfur dioxide levels within cabinets was tested by paired comparisons between the 24 and 72 hr EqpH readings for cabinets that had significant levels of sulfur dioxide. In the cases of the GSC and CMN cabinets, EqpH readings at 72 hr were significantly lower than readings at 24 hr. In other cabinets, including ROM cabinets with significant levels of sulfur dioxide, there was no significant difference in EqpH readings at 24 and 72 hr. Consequently, with the exception of those CMN and GSC cabinets that had significant levels of sulfur dioxide, the 24 and 72 hr readings of EqpH were averaged to obtain the best possible value for EqpH.

The mean EqpH data for each collection are shown in Table 6. These data show that the GSC collection is slightly less acidic than the CMN collection and that the ROM collection is significantly more acidic than the other two. It is interesting

Figure 4.   Mean equilibrium pH, within cabinets, at 24 hr. CMN, Canadian Museum of Nature; GSC Geological Survey of Canada; ROM, Royal Ontario Museum.

to note that the difference in apparent carboxylic acid concentration between the most and least acidic collections is only about half an order of magnitude.

Within the ROM collection, EqpH was examined for different cabinet types. The results are shown in Table 7. The case types present are type 2 (old varnished cherry wood), type 3 (unfinished plywood), type 4 (unfinished plywood but with varnished doors), and type 5 (old cherry wood cabinets with new plywood drawers). For type 5 cabinets, the 90% confidence interval is large because of the small sample size, consequently, no conclusions can be drawn comparing these to the other types of cabinets. However in considering the other three types of cabinet,

Table 6.   Comparison of equilibrium pH measurements from all cabinets in each collection.

	Construction	Mini-mum pH	Maxi-mum pH	Mean pH	Stan-dard devia-tion	Num-ber of obser-vations	90% Confi-dence interval	Equiv-alent ppm acetic acid
CMN	Metal cabinet and drawers, well-sealed	3.6	4.8	4.27	0.25	279	4.25–4.30	1
GSC	Metal cabinet and wooden drawers, not sealed	4.1	4.6	4.36	0.09	109	4.35–4.38	0.8
ROM	Wooden cabinets and drawers, well-sealed	3.0	4.4	3.58	0.13	334	3.57–3.59	3.5

CMN, Canadian Museum of Nature; GSC, Geological Survey of Canada; ROM, Royal Ontario Museum.

Table 7. Combined 24 and 72 hr mean equilibrium pH for differing cabinet types.

Case type	Description	Mean pH	Standard deviation	Number of observa- tions	90% Confidence interval
2	old varnished cherry wood	3.61	0.13	194	3.59–3.63
3	unfinished plywood	3.52	0.07	102	3.51–3.53
4	unfinished plywood with varnished doors	3.57	0.08	26	3.54–3.60
5	old cherry wood cabinets with new plywood drawers	3.68	0.28	12	3.50–3.85

the mean EqpH is significantly higher for the cherry wood cabinets, and the 90% confidence interval range does not overlap the other two ranges. The mean EqpH for the plywood cabinets with varnished doors is significantly lower than that of the cherry wood cabinets; it is slightly, but not significantly, higher than that of the unfinished plywood cabinets. The unfinished plywood cabinets have the lowest mean EqpH. From these results, it would appear that the age, type, and surface treatments of the wood all effect the EqpH inside the cabinets. The addition of varnish to part of the internal structure of the cabinet appears to have reduced emissions of carboxylic acids.

Equilibrium pH readings as a function of cabinet position within the systematic ordering, for each of the three collections is shown in Figure 5. The Geological

Figure 5. Equilibrium pH readings, within cabinets, as a function of cabinet position within the systematic ordering within each collection. CMN, Canadian Museum of Nature; GSC, Geological Survey of Canada; ROM, Royal Ontario Museum. See Table 5 for legend.

Table 8. Odours recorded for sampled cabinets.

Code	Odour	Code	Odour	Code	Odour
1.0	None	2.0	None	3.0	None
1.1	Musty (very slightly acrid)	2.1	Polish (very very faintly woody)	3.1	Strange/unidentified
1.2	Slightly acrid	2.2	Sweet (very faintly woody)	3.2	(not used)
1.3	Acrid	2.3	Sickly (faintly woody)	3.3	Sulfurous
1.4	Very acrid	2.4	Woody	3.4	Rubbery
		2.5	Very woody		

Survey of Canada collection has the simplest distribution to interpret. Equilibrium pH values are closely distributed about the mean value of 4.4. This is only slightly, and probably insignificantly, lower than the value of 4.5 recorded for the room air and is a result of the high rate of air exchange between the cabinets and the room as a result of a lack of gasketting.

The Canadian Museum of Nature collection shows EqpH values scattered widely on either side of the mean value of 4.3. The mean value for the room air was 4.5 indicating that the cabinets are on average slightly more acidic than the room. The wide scatter and difference between EqpH in the room and in the cabinets indicates that there is a low rate of air exchange between the cabinets and the room. Because the mean is near the ambient EqpH, the EqpH in these cabinets is not greatly affected by the materials of the cabinets themselves. However, because the cabinets are so well sealed, their internal atmosphere is strongly affected by their mineral contents. Some general observations can be made regarding the distribution of EqpH values in the CMN collection. EqpH in native elements cabinets are near the mean. In the sulfides, and especially the disulfides, EqpH values lower than the mean appear—these are probably affected by SO_2 from oxidising pyrite in specimens. Equilibrium pH values in the halides, miscellaneous oxysalts, and silicates are nearly evenly distributed about the mean for the collection. Most importantly, many cabinets holding oxides or carbonates had EqpH higher than the mean indicating that these specimens are absorbing or reacting with acidic vapours.

Ninety percent of the cabinets in the Royal Ontario Museum collection have EqpH values in the range 3.5 to 3.7. This is because of the release of acidic vapours from the wooden components of cabinets and drawers combined with relatively air-tight construction and closure. The specimens in this collection appear to have less effect on the EqpH of the air than the specimens in the CMN collection because of the logarithmic nature of the pH scale described above. In the ROM collection five cabinets had EqpH values below the range of 3.5–3.7. Two of these cabinets contained the entire holdings of specimens labelled marcasite, a third cabinet also contained sulfide minerals, and the remaining two cabinets were located in a separate small room where the room air EqpH was 3.6 (in contrast to the collection room where the EqpH of the air was 4.5). Five cabinets had interior EqpH values higher than 4.0. Two of these contained carbonates as the primary species, two had species commonly associated with calcite

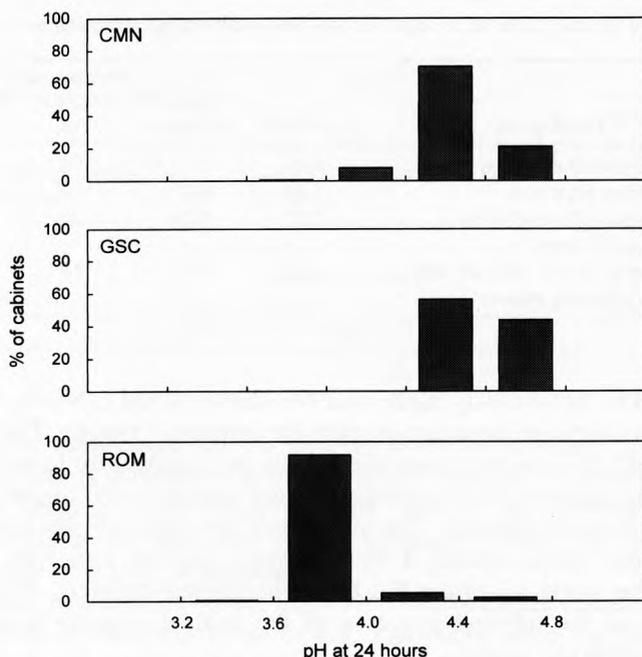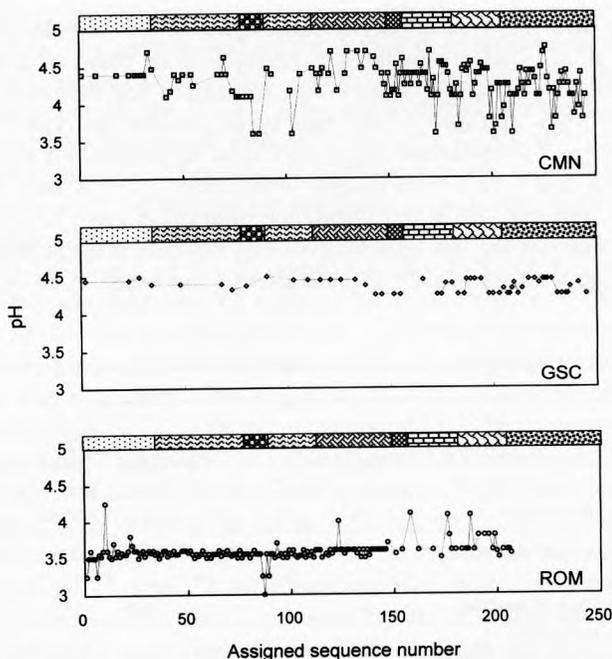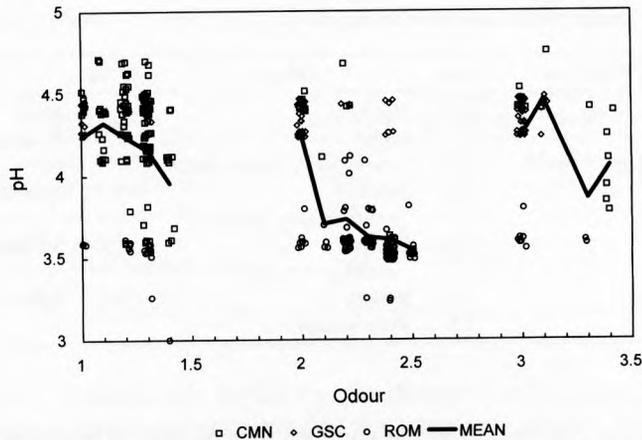Figure 6. Equilibrium pH readings as a function of odour within cabinets. CMN, Canadian Museum of Nature; GSC, Geological Survey of Canada; ROM, Royal Ontario Museum.

matrices, and the fifth contained phosphates including highly alkaline tribasic salts. These results indicate that both the specimen contents of the cabinets and the collection room EqpH can affect the EqpH within these cabinets.

Because odour in cabinets was recorded (see Table 8), this frequently employed subjective measure could also be compared with the EqpH values (Fig. 6). Only two of the three kinds of odour observed had sufficient data to allow assessment of the utility of odour as an indicator of EqpH levels. These were the group 1 (acrid odour) cabinets in the CMN collection and the group 2 (woody odour) cabinets in the ROM collection. As indicated by the mean EqpH curves in Figure 6, the presence of either an acrid or woody odour correlated with a tendency to low EqpH. Increasing acrid odour correlates with decreasing EqpH over most of the range recorded. However, the scatter of individual data points indicates that there are many exceptions to this tendency. The presence of a woody odour, in contrast, is a relatively reliable indicator of low EqpH.

A second, independent indicator of carboxylic acid concentrations was obtained from the results for the lead foil test strips (Fig. 7 and Table 9). These data indicate that the CMN collection has the lowest overall concentration of lead-corroding acids equivalent to 0.8 ppm acetic acid. The GSC collection was intermediate, equivalent to 1.9 ppm acetic acid, and the ROM had the highest average concentration, equivalent to 6.5 ppm acetic acid. In the GSC and ROM collections these results indicate a higher equivalent acetic acid concentration than that indicated by the pH test strips (compare Tables 9 and 6). Figure 8 shows the data for acetic acid concentration plotted against the measured EqpH. The line depicts the expected relationship based on Tétreault's (1992) study of the response of pH strips to specific concentrations of acetic acid. The overall correlation of these two indicators is good. However at low EqpH, data points lie mostly above the calibration curve. This indicates that the lead foil is corroding more rapidly than would be expected based only on a consideration of an acetic acid concentration corresponding to the EqpH. This may result from formaldehyde emissions from the adhesive in the plywood making up the collection furniture, although little
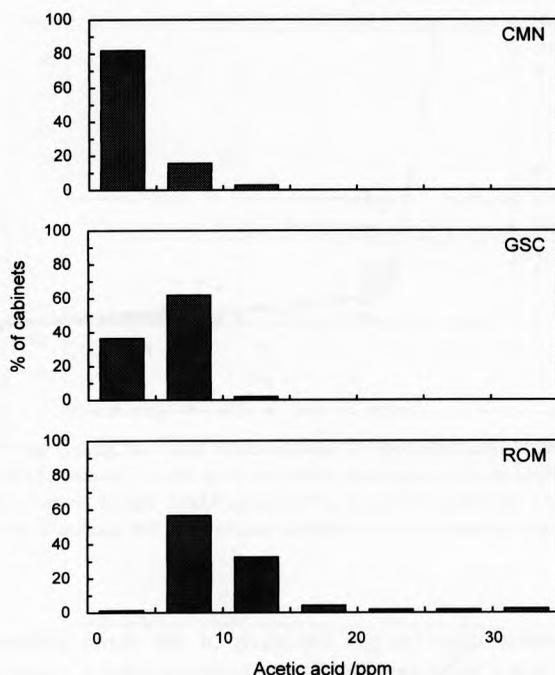
Figure 7.   Equivalent acetic acid concentrations, within cabinets, as indicated by lead foil after 3 mo of exposure in each collection. CMN, Canadian Museum of Nature; GSC, Geological Survey of Canada; ROM, Royal Ontario Museum.

reaction would be expected from plywood (Grzywacz and Tennent 1994); formaldehyde might react with the lead but, being a very weak acid, would not have any significant effect on EqpH. The statistically higher result for acetic acid concentration in the GSC, as compared with the CMN may, in part, be a result of the effect of a higher average relative humidity in those cabinets on the sensitivity of the lead foil. Finally, a combination of acidic pollutants may have a synergistic effect on the corrosion of lead but not an additive effect on EqpH.

Sulfur Dioxide

The presence of sulfur dioxide was indicated by the palladium chloride test strips, the copper foil, and the sulfite ion test paper. Of these, the sulfite ion test paper was least subject to interference and provided the only reliable record.

Table 9.   Comparison of the apparent acetic acid concentration based on the 3-mo readings from the lead foil test strips from all cabinets in each collection.

	Mean ppm acetic acid	Standard deviation	Number of observations	90% Confidence interval
CMN	0.8	1.7	148	0.5–1.0
GSC	1.9	1.6	55	1.5–2.4
ROM	6.5	6.1	170	5.6–7.4

CMN, Canadian Museum of Nature; GSC, Geological Survey of Canada; ROM, Royal Ontario Museum.
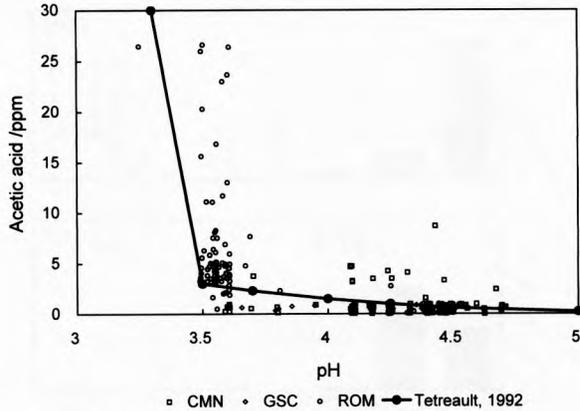
Figure 8. Carboxylic acid concentration as measured on lead foil after 3 mo as a function of pH. The line indicates the relationship expected based on data from Tétreault (1992). CMN, Canadian Museum of Nature; GSC, Geological Survey of Canada; ROM, Royal Ontario Museum. Data points have been randomly dispersed around the measured values to better illustrate distribution of measurements.

Sulfur dioxide concentration ranges for each of the three collections are shown in Figure 9. Both the CMN and ROM collections had a significant number of cabinets containing measurable sulfur dioxide levels. The GSC collection had only a single cabinet with a measurable sulfur dioxide concentration, and this concen-
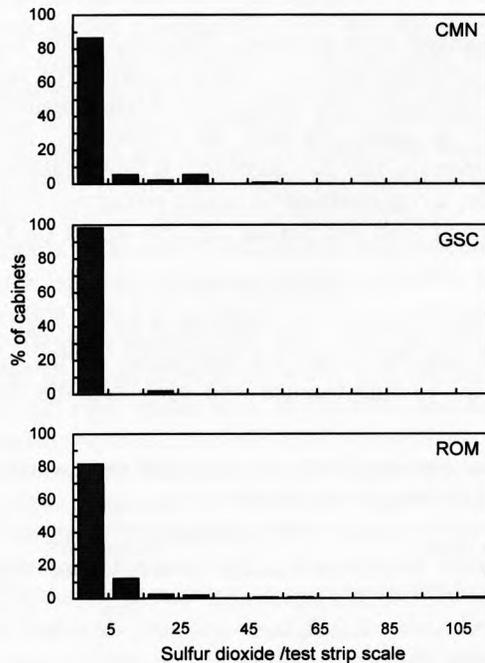
Figure 9. Sulfur dioxide concentration as indicated by sulfite ion test paper after 72 hr. CMN, Canadian Museum of Nature; GSC, Geological Survey of Canada; ROM, Royal Ontario Museum.
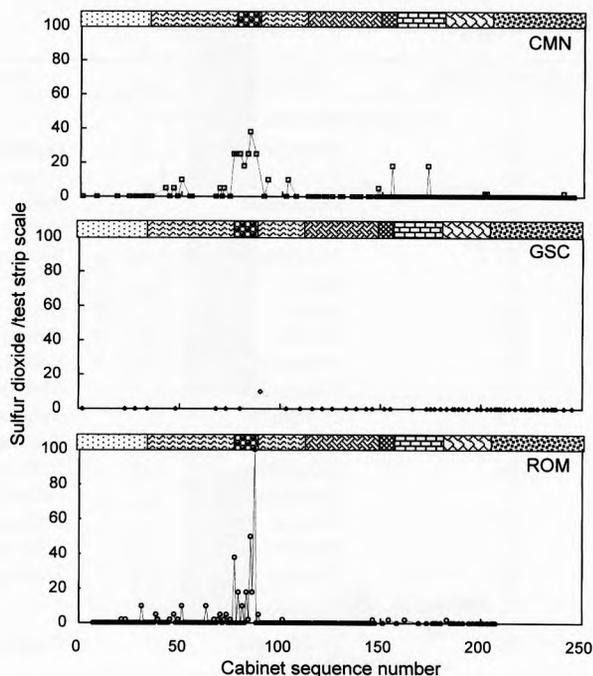
Figure 10.   Sulfur dioxide concentrations as a function of cabinet sequence. See Table 5 for legend. CMN, Canadian Museum of Nature; GSC, Geological Survey of Canada; ROM, Royal Ontario Museum.

tration was only a fraction of levels measured in the other collections. The CMN collection contained a number of cabinets with measurable sulfur dioxide levels, but the highest level recorded was considerably lower than the highest level at the ROM. This was true despite the fact that the CMN cabinet with the highest concentration was 90% full, whereas the two ROM cabinets with higher concentrations were only 60 and 40% full.

Sulfur dioxide concentrations in the GSC collection cabinets were thought to be low because of the lack of gasketting and consequent high rates of air exchange. In addition, because a single cabinet contains a wide range of species in the systematic ordering of this collection, there were no cabinets only containing iron disulfide minerals. In the other collections, it was these cabinets which had the highest sulfur dioxide levels.

There are several possible reasons why higher concentrations were recorded in the ROM collections than in the CMN collection: (1) the higher relative humidity levels actually measured in these cabinets would lead to higher rates of sulfide mineral oxidation. (2) Silica gel, used for relative humidity stabilisation in the CMN collection cabinets, is acting as a pollutant scavenger, as evidenced by an amber discolouration of its surface. There is no silica gel in the ROM cabinets. (3) The ROM cabinets may be more tightly sealed than those of the CMN. (4) Prior to the move to a new building, collections at the ROM were housed in areas with a wide range in relative humidity levels (Waddington and Rudkin 1983). Reactive specimens may be continuing to oxidise at an accelerated rate (Waller

Table 10. Cabinets containing significant levels of sulfur dioxide.

SO_2	Sequence number	Class start	Class end
		Canadian Museum of Nature	
38	28	Bravoite	Gersdorffite
25	24	Pyrite	Pyrite
25	23	Getchellite	Pyrite
25	25	Pyrite	Pyrite
25	29	Marcasite	Marcasite
25	27	Pyrite	Pyrite
18	26	Pyrite	Pyrite
18	58	Calcite	Calcite
18	76	Dolomite	Dolomite
10	31	Glaucodot	Molybdenite
10	15	Sphalerite	Sphalerite
10	33	Tennantite	Tetrahedrite
5	19	Talnakhite	Valleriite
5	21	Covellite	Wakabayashilite
5	13	Galena	Galena
5	11	Bornite	Galena
5	51	Fluorite	Fluorite
		Geological Survey of Canada	
10	90	Frohbergite	Canfeldite
		Royal Ontario Museum	
100	86	Marcasite	Marcasite
50	84	Pyrite	Bravoite
38	76	Pyrite	Pyrite
18	78	Pyrite	Pyrite
18	85	Vaesite	Marcasite
18	82	Pyrite	Pyrite
10	62	Millerite	Ruthenarsenite
10	50	Sphalerite	Sphalerite
10	31	Sulfur	Sulfur
10	80	Pyrite	Pyrite
5	38	Chalcocite	Chalcocite
5	88	Gersdorffite	Gersdorffite
5	46	Galena	Galena
5	72	Orpiment	Stibnite
5	69	Picotpaulite	Stannite

1990). Of these factors, it is not clear which has the major effect on sulfur dioxide levels.

Sulfur dioxide concentrations as a function of cabinet sequence are shown in Figure 10. The GSC collection had only one cabinet with a detectable level of sulfur dioxide, this contained sulfide and sulfosalt specimens. Distribution of sulfur dioxide concentrations was similar in both the CMN and ROM collections. In both collections, the highest levels were recorded in cabinets in the sequence 76–87. Table 10 shows these to be the cabinets containing the iron disulfide minerals, pyrite, and marcasite. A second group of cabinets having measurable levels of sulfur dioxide was found in the cabinet number range 38–50. These cabinets contained sulfide minerals such as chalcocite, bornite, galena, sphalerite,
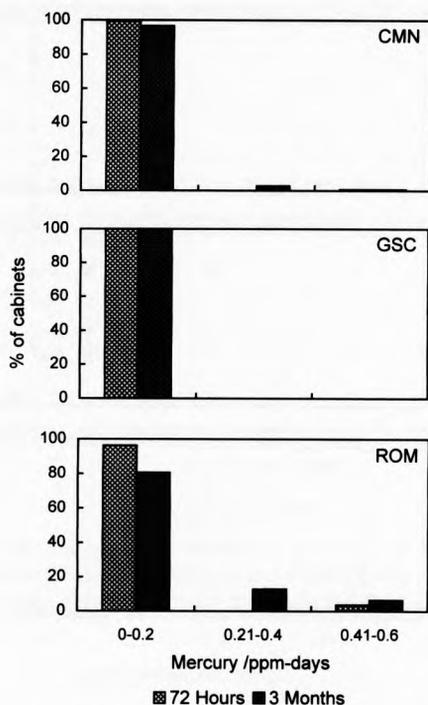
Figure 11.   Mercury concentrations as indicated by the palladium chloride test paper at (a) 72 hr and (b) 3 mo. CMN, Canadian Museum of Nature; GSC, Geological Survey of Canada; ROM, Royal Ontario Museum.

etc. Here, the oxidation of microcrystalline pyrite or marcasite in the matrices of specimens in these cabinets is probably responsible for the sulfur dioxide.

In calcite- and dolomite-containing cabinets (CMN 155 and 173), detectable levels of sulfur dioxide are generated by microcrystalline pyrite or marcasite in the matrices of specimens. The identification of high levels of inorganic acid pollutants in cabinets that contain carbonate species is of interest, and should be of concern to conservators, because sulfur dioxide is well known to cause damage to carbonate rocks (Thomson 1986).

A value of 2 was assigned to sulfite test strips that appeared to be very slightly pink but not sufficiently pink to be assigned the intermediate value of 5 (see section entitled, *Reading Results from Test Strips*). This was a difficult decision because all sulfite test strips developed a slight beige cast when moistened with the glycerol solution. The contents of the three CMN cabinets with this recorded level were visually inspected for the presence of specimens containing oxidising pyrite. Only one of these cabinets, the quartz containing cabinet, had such a specimen. It was concluded that a value of 2 is an uncertain finding. Cabinets for which this value was recorded may or may not contain more than trivial amounts of sulfur dioxide.

The sulfite ion test strips as employed in this study effectively identified sources of sulfur dioxide throughout the collections. They could, therefore, be used to locate specimens undergoing pyrite oxidation.

Figure 12.   Mercury vapour concentrations as a function of cabinet sequence, measured at 72 hr using palladium chloride paper. See Table 5 for legend. CMN, Canadian Museum of Nature; GSC, Geological Survey of Canada; ROM, Royal Ontario Museum.

Mercury

Mercury concentrations within cabinets, as indicated by the palladium chloride test paper, are shown in Figure 11. The range over both 72 hr and 3 mo is similar. As discussed in the previous section on calibration of the palladium chloride test strips, the highest value documented is 0.59 ppm-days of mercury. At both exposure durations information about high mercury concentrations is lost as a result of the saturation effect detailed in the section entitled, *Palladium Chloride Paper.* At low concentrations, there is uncertainty resulting from discoloration of the test strips by sulfide and other trace pollutants. In retrospect, it would have been better to monitor mercury at 24 hr. Because the reaction involves simple pigmentation of the paper with metallic palladium and the palladium chloride reagent is unlikely to react fully, the darkening of the test paper is interpreted as a simple linear function of both concentration and time. In addition, because the time scale for the testing, when read at 72 hr, is similar to the time employed for calibration, mercury concentrations in cabinets could be compared to those obtained in the calibration experiment, assuming reciprocity between concentration and time. However, once the response of a test strip reaches the saturated dark grey colour, only a minimum mercury concentration can be ascerta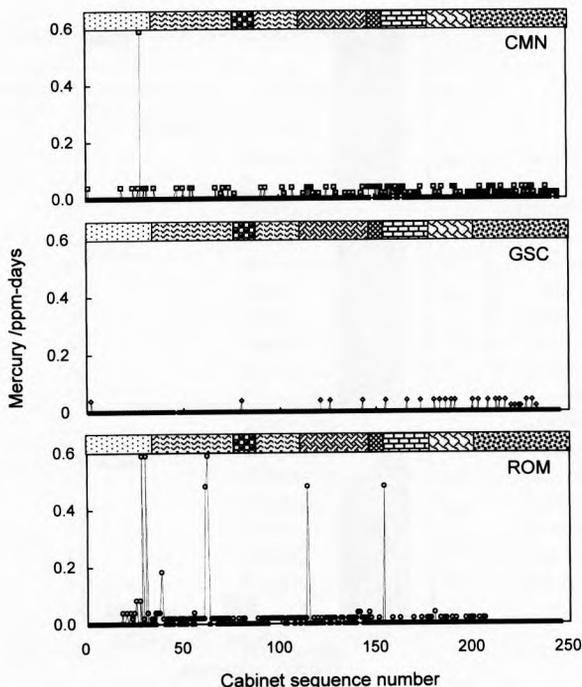ined. Mercury vapour concentrations as a function of cabinet sequence, measured at 72 hr, using the palladium chloride paper, are shown in Figure 12.

In addition to darkening on exposure to mercury vapour, the palladium chloride darkened on exposure to low levels of reduced sulfur gas, as discussed earlier.
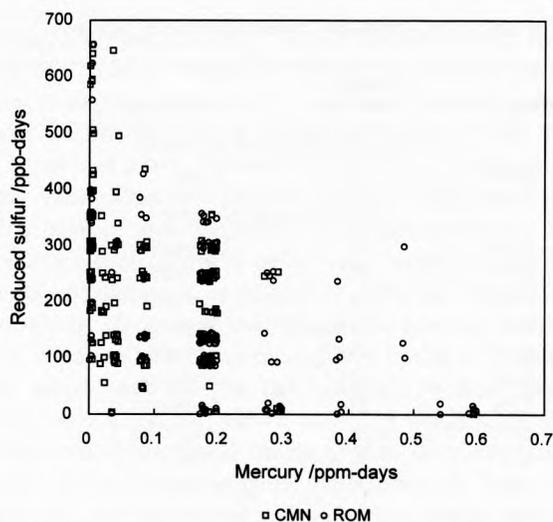
Figure 13.   Reduced sulfur-bearing gas concentration as a function of mercury concentration within cabinets in the CMN (Canadian Museum of Nature) and ROM (Royal Ontario Museum) collections. Data points have been randomly dispersed around the measured values to better illustrate the distribution of measurements.

The palladium chloride paper often developed a grey flecking rather than a uniform grey colour after 72 hr of exposure. This response resembled the mercury calibration photographs in colour. However, the colour was unevenly distributed, better resembling the appearance of the hydrogen sulfide calibration photographs. Reactions of this kind were assigned a reading for both mercury and reduced sulfur. Interestingly, when mercury and reduced sulfur data from the ROM and CMN collections were considered together, high levels of mercury were never associated with high levels of reduced sulfur gases. Figure 13 shows ppb-days reduced sulfur gas plotted against ppm-days mercury, measured at 3 mo, for both collections. Few cabinets, of the 301 cabinets sampled, had reduced sulfur and mercury concentrations lying above a triangular region bounded by both high reduced sulfur and high mercury vapour concentrations. This may reflect the limit of the product of mercury and hydrogen sulfide fugacities above which mercury sulfide would form. This suggests, that at least some of the moderate levels of mercury detected, 0.1–0.3 ppm-days, represent actual levels of mercury vapour. Unfortunately, at these levels, it is not known which readings might be reliable and which might be spurious.

This confusion sets a lower limit on mercury vapour detection. The exact level of this lower limit is uncertain but, for the purposes of this study, it was set at an accumulated reading after 3 mo of 0.3 ppm-days. It is certain that readings higher than this result from an appreciable presence of mercury vapour. Cabinets, for which readings lower than this were obtained, might still contain appreciable levels of mercury vapour, but these levels are certainly at least 10 times lower, and perhaps several magnitudes lower, than the levels measured in cabinets containing mercury specimens. Using the 0.3 ppm-day equivalent response after 3 mo as the criterion for selecting cabinets with high mercury concentrations, the

Table 11. Cabinets containing >0.3 ppm-day levels of mercury vapour.

PdCl at 3 mo	PdCl at 72 hr	Sequence number	Class start	Class end
		Canadian Museum of Nature		
0.59	0.59	28	Lead	Chukhrovite
		Royal Ontario Museum		
0.59	0.59	62	Cinnabar	Millerite
0.59	0.59	30	Bismuth	Tellurium
0.59	0.59	28	Auricupride	Lead
0.59	0.48	114	Tenorite	Corundum
0.59	0.48	61	Cinnabar	Cinnabar
0.59	0.48	154	Nadorite	Perylite
0.54	0.08	27	Copper	Copper
0.54	0.04	35	Argentite	Argentite
0.48	0	7	Platinum	Rhodium
0.48	nd	2	Gold	Gold
0.48	nd	4	Gold	Gold
0.38	0.18	38	Chalcocite	Chalcocite
0.38	0.02	113	Periclase	Tenorite
0.38	0	17	Silver	Silver
0.38	0	15	Silver	Silver
0.38	0	14	Silver	Silver
0.38	nd	1	Gold	Gold
0.38	nd	5	Gold	Opal

nd, not determined.

three collections were found to have the following numbers of cabinets with detectable mercury vapour: CMN—1, GSC—0, ROM—18.

The single cabinet at CMN contained the native mercury specimens. Although the majority of these specimens were sealed in glass jars, the few nonenclosed specimens were sufficient to give the maximum response on the test paper after 72 hr. This response is thought to indicate a mercury concentration in the cabinet of at least one-twelfth of the equilibrium vapour pressure for mercury at room temperature. Hence, the concentration is thought to be on the order of 1 ppm.

The situation at the ROM is more complex (Table 11). Three cabinets showed the full response possible after 72 hr, only one of these cabinets, number 15, contained specimens with visible free mercury. A second contained cinnabar specimens from a locality from which native mercury is known to occur but no mercury was visible on the specimens. The third contained only specimens of the species bismuth, selenium, and tellurium. For both of these latter cabinets it is thought that much of the mercury vapour may be contained in the wood of the cabinet walls and drawers that had been absorbed at a time when the cabinets and/or drawers contained native mercury bearing specimens. Determining whether wood components in isolation emit mercury could test this hypothesis. Three other cabinets showed full responses after 3 mo of exposure but responses equivalent to only 0.5 ppm-day after 72 hr exposure. All of these cabinets held a mercury-containing species and the mercury vapour probably derives from trace amounts of native mercury associated with these specimens.

For many of the cabinets that show a lesser, but still significant, concentration

of mercury, the source of the mercury vapour is less clear. In the cabinet containing the species periclase to tenorite (Table 11, cabinet 113), it is suspected that a mercury-containing specimen had previously been housed in this cabinet but had been moved during expansion of the collection to the next cabinet in the sequence (Table 11, cabinet 114). Cabinet 114 was one of the six cabinets showing a full response after 3 mo. This cabinet did contain visible mercury on a specimen of montroydite. The present arrangement dates from the move into new premises in 1981. Two cabinets which showed detectable levels of mercury were located apparently five to six cabinets out of sequence with the current location of native mercury. However, about 10 yr ago, the specimens housed in the first six cabinets were moved into a separate small room and the entire collection was shifted to fill the space. This would account for the anomaly in that these cabinets, in all probability, formerly contained the native mercury specimens. For the remaining cabinets, a common characteristic of many is that they contain native elements known to be capable of forming amalgams with mercury. It is possible that much of the mercury detected is being desorbed from specimens removed from exhibit in 1981 following about 20 yr on display in a single, long display case that also included mercury specimens. Monitoring the atmosphere around individual specimens and comparing results with documentation describing the exhibit history of specimens could test this hypothesis.

Reduced Sulfur Gasses

The test papers and metal foils used in the experiment yielded 11 data points for each cabinet that were initially thought to provide some measure of reduced sulfur gas concentrations. These were the lead acetate papers, buffered and unbuffered at 72 hr and 3 mo, the palladium chloride paper, silver and copper at 72 hr, and the palladium chloride paper, silver, copper, and lead at 3 mo. Some of these acted as neither monitors nor dosimeters but were intermediate in their behaviour. One of the best examples of this type of behaviour was the unbuffered lead acetate paper. This paper exhibited colour responses equivalent to low concentration-time dosages after 72 hr but either faded, stayed the same, or developed higher concentration colours by the time the 3 mo reading was taken. This variation in behaviour is thought to depend on the concentration of reduced sulfur gas, the sulfur dioxide concentration, and the EqpH of the cabinet.

All of the indicators were subject to interference from at least one other factor that was variable between cabinets. The most extreme example of this was the effect of carboxylic acids on the lead foil after 3 mo exposure. Where these acids significantly corroded the lead, any reaction to reduced sulfur gasses was unnoticeable. None of the indicators used were entirely reliable for indicating reduced sulfur gas concentrations in cabinets. A general review of the data showed that the readings from the 3-mo exposure of silver foil provided the most useful data. This was the only indicator capable of discriminating between cabinets with virtually no reduced sulfur gas and those with a low level similar to the room atmosphere (Fig. 14). All other indicators recorded 0 ppb-day equivalent of hydrogen sulfide (i.e., showed no response) for a large proportion of the cabinets. Consequently, the 3-mo reading of the silver foil was considered to provide the best data for this study and is used below.

Distributions of reduced sulfur gas concentration, as indicated by silver foil
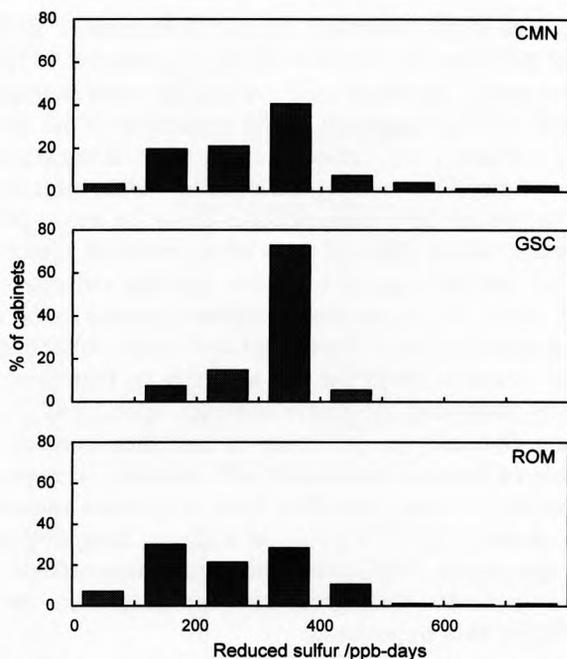
Figure 14.   Reduced sulfur-bearing gas concentrations as indicated by silver foil observed after 3 mo
of exposure. CMN, Canadian Museum of Nature; GSC, Geological Survey of Canada; ROM, Royal
Ontario Museum.

observed after 3 mo of exposure are shown in Figure 14. For all three collections,
concentrations are approximately normally distributed about means of approxi-
mately 200 ppb-days. Both the ROM and CMN collections showed greater de-
viations from the mean, ranging from 0–650 ppb-days, whereas the GSC collec-
tion ranged only from 100–350 ppb-days. This difference is attributed to the
relative leakiness of the GSC cabinets compared to those in the other collections.

Figure 15 shows the concentration distribution of reduced sulfur gas as a func-
tion of cabinet sequence number. For all three collections, recorded values show
considerable scatter and are centred somewhat above the value recorded for the
collection room. For example, the mean for the CMN cabinets was 234 ppb-days
compared to a room measurement of 135 ppb-days; the mean for the GSC cabinets
was 248 ppb-days compared to a room measurement of 95 ppb-days; and the
mean for the ROM cabinets was 179 ppb-days compared to a room measurement
of 135 ppb-days. Even considering the uncertainty of individual measurements of
room concentrations, which may be as high as ±50%, we conclude that mean
concentrations within cabinets are significantly higher than room concentrations.
This indicates that the cabinet contents, over all, are a net producer of reduced
sulfur gas. This is not surprising considering that reduced sulfur is a common
constituent of mineral species. It is not known why the GSC showed the greatest
difference between cabinets and room concentrations despite the relatively high
leakage rate of those cabinets. Because only a single test strip was deployed to
measure the room concentration, this single measurement may, per chance, have
been lower than the true average concentration in the room.
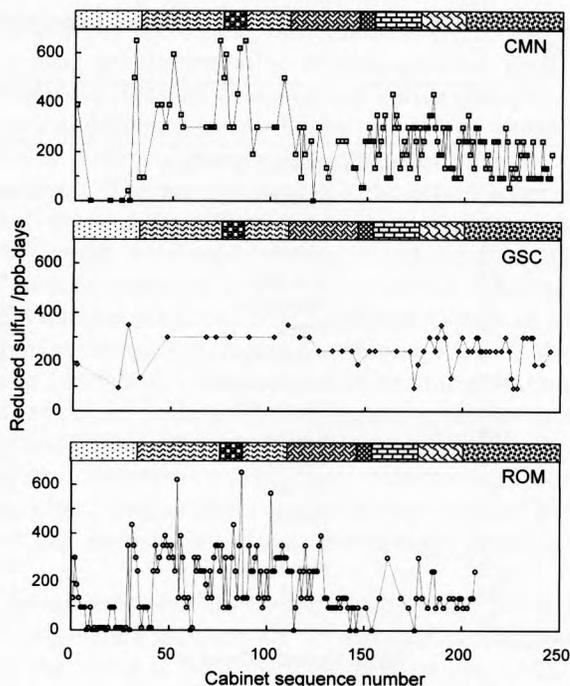
Figure 15.   Reduced sulfur-bearing gas concentrations as indicated by silver foil after 3 mo exposure as a function of cabinet sequence number. See Table 5 for legend. CMN, Canadian Museum of Nature; GSC, Geological Survey of Canada; ROM, Royal Ontario Museum.

Currently, there is little information regarding the capacity of different mineral species to release or react with reduced sulfur gasses. The overall complexity of the relationship between reduced sulfur gasses and cabinet sequence number suggests that many cabinets may contain species that are actively either emitting or reacting with reduced sulfur gasses. In an attempt to identify some of the most reactive mineral species with respect to reduced sulfur gasses, cabinets having greater than 400 ppb-days or less than 50 ppb-days of reduced sulfur gasses were identified and are shown in Table 12. Most cabinets with high levels of reduced sulfur gasses, as indicated by the silver foil strips after 3 mo exposure, contained either native sulfur or oxidising disulfide species. Cabinets with low levels of reduced sulfur, as indicated by the silver foil after 3 mo of exposure contained native silver, copper, or mercury, or manganese oxide species. In cabinets containing native metallic elements, these specimens must be reacting rapidly enough to keep the reduced sulfur gas concentration negligible. Many of the manganese oxide species in the CMN collection were in the form of dendrites in fine-grained, porous limestone matrices. Possibly these limestone matrices are also scavenging reduced sulfur, but the mechanism for this is unknown. We have already shown that reduced sulfide levels are always low in cabinets with high levels of mercury. This proved to be the case in cabinet 28 at the CMN and cabinet 61 at the ROM.

Of particular interest, are the pronounced peaks and troughs in reduced sulfur gas concentrations indicated throughout the native element and sulfide sections of collections (Fig. 15). The most extreme examples of this at both the CMN and

Table 12. Cabinets having >400 ppb-days or 0 ppb-days of reduced sulfur-bearing gas as indicated by silver foil after 3 mo.

ppb-day H₂S	Sequence number	Class start	Class end
		Canadian Museum of Nature	
650	31	Sulfur	Sulfur
650	87	Marcasite	Marcasite
650	74	Stibnite	Stibnite
620	84	Bravoite	Gersdorffite
595	50	Sphalerite	Sphalerite
595	77	Pyrite	Pyrite
500	30	Antimony	Tellurium
500	107	Seligmannite	Livingstonite
500	76	Getchellite	Pyrite
435	163	Calcite	Calcite
435	184	Gypsum	Gypsum
435	83	Pyrite	Pyrite
0	18	Silver	Silver
0	8	Silver	Silver
0	122	Pyrolusite	Todorokite
0	28	Lead	Chukhrovite
0	24	Copper	Copper
		Royal Ontario Museum	
650	87	Marcasite	Marcasite
620	54	Sphalerite	Sphalerite
565	102	Jordanite	Tetrahedrite
435	83	Pyrite	Pyrite
435	31	Sulfur	Sulfur
0	143	Marokite	Hollandite
0	176	Malachite	Malachite
0	114	Tenorite	Corundum
0	154	Nadorite	Perylite
0	8	Silver	Silver
0	27	Copper	Copper
0	18	Silver	Silver
0	61	Cinnabar	Cinnabar
0	13	Silver	Silver
0	30	Bismuth	Tellurium
0	146	Ferrocolumbite	Franconite
0	11	Silver	Silver

ROM are in cabinets 30 and 31 where metallic native elements are stored adjacent to native sulfur. Other examples are less easily explained and require further investigation.

SUMMARY AND CONCLUSIONS

Low-cost pollutant indicators for use in storage cabinets were found or developed. Although none of these were capable of precise quantitative results, it was possible to achieve qualitative or semiquantitative indications for each of the pollutants of interest. Some of these indicators are likely to be of interest in other applications. For example, the palladium chloride strip was useful for identifying mercury vapour and will have importance for reasons of health and safety. It may

also find an application in zoological and botanical collections in which mercuric chloride has been used as a preservative (Hawks and von Endt 1990). The sulfite ion test strips effectively identified sources of sulfur dioxide and could be used to locate specimens undergoing pyrite oxidation.

The three types of storage cabinets were found to have a significant effect on internal pollutant levels. The air tightness of cabinets, as determined by construction method, door fit, and the effectiveness of gaskets had a major effect on pollutant levels observed. As would be expected (Michalski 1994), for most pollutants, the less air-tight cabinets at the GSC showed relatively little difference between room and cabinet pollutant concentrations except in cabinets containing clusters of pollutant emitting or pollutant scavenging mineral specimens. This was the only collection for which room air pollutant concentration levels appear to have a significant effect on pollutant concentration levels in cabinets. Well-sealed cabinets demonstrated internal pollutant concentration levels differing greatly from room pollutant concentration levels and related much more directly to cabinet contents. Therefore, well-sealed cabinets protect cabinet contents from external pollutants but can increase specimen interactions through internal pollutant interactions.

Well-sealed wooden cabinets contribute to an overall lower internal EqpH of internal air, especially where cabinets are constructed of unfinished wood, but this is true even when the wood is varnished and several years old. Wooden cabinets also appear to be able to absorb and desorb mercury vapour from mercury-containing specimens.

The distribution of pollutant concentration levels within the order of systematic mineral collections was evaluated. Some mineral species were found to be major sources of pollutants, whereas others act as sinks by reacting with pollutants. Minerals that emit pollutants may do so by simple sublimation or evaporation, as in the cases of sulfur or mercury, or by chemical reaction, as in the formation of sulfur dioxide from the oxidation of pyrite. Minerals emitting pollutants by sublimation or evaporation should be sealed to ensure their preservation as well as reducing their effects as a pollutant source. Pollutant scavengers, such as activated charcoal or potassium permanganate, should not be enclosed with these specimens. The use of scavengers should only be considered for those minerals that emit a pollutant as the result of a thermodynamically irreversible chemical reaction, such as oxidising pyrite.

In summary, the major conclusions of this work are that pollutant concentration levels in mineral collection cabinets are less dependent on room-air pollutant concentration levels than on the cabinet materials and mineral species present. Mineral species may be either emitters or absorbers of pollutants. Care must be exercised to avoid housing species that absorb and emit the same pollutant within a single well-sealed cabinet.

FUTURE RESEARCH

This project was undertaken to establish an initial assessment of the pollutant concentration levels in systematic mineral collections. Many interesting and potentially fruitful possibilities for further investigations have been identified as a result of this work. They include the following topics: (1) investigation of pollutant concentration levels within individual cabinets to establish their spatial var-

iation. (2) Investigation of diverse single mineral species as pollutant sources or sinks. In particular, identification of sulfide species as net producers or consumers of reduced sulfur gasses. (3) Determination of the rates of damage, in terms of loss in value over time, for susceptible specimens exposed to pollutant gas concentration levels observed in cabinets. (4) Estimation of the magnitude of risk to mineral collections resulting from these internal pollutants. (This has now been completed [Waller 1999].) (5) Calibration of each of the test strips under concentration-time conditions comparable to those employed in the survey process. (6) Improved characterisation of the palladium chloride test strip for mercury vapour, including the determination of shelf life and optimum methods for calibration.

LITERATURE CITED

Agnew, N. 1981. The corrosion of egg shells by acetic acid vapour. Institute for the Conservation of Cultural Material Bulletin 7(4):3–9.

Andrew, K.J., J. Tétreault, and R.R. Waller. 1993. A survey of pollutant concentrations in mineral collection cabinets. Scottish Society for Conservation and Restoration 4(1):13–15.

Bailey, S.W. (ed.). 1988. *Hydrous Phyllosilicates (exclusive of micas)*. Reviews in Mineralogy 19, Mineralogical Society of America, Washington. 725 pp.

Blackshaw, S.M. and V.D. Daniels. 1979. The testing of materials for use in storage and display in museums. The Conservator 3:16–19.

Brimblecoombe, P. 1989. *The chemistry of museum air*. Preprints of the meeting on Environmental Monitoring and Control, Scottish Society for Conservation and Restoration and the Museums Association. 15–16 March, Dundee, Scotland. Pp. 56–63.

Brimblecoombe, P., D. Shooter, and A. Kaur. 1992. Wool and reduced sulphur gases in museum air. Studies in Conservation 37(1):53–60.

Clarke, S.G. and E.E. Longhurst. 1961. The corrosion of metals by acid vapours from wood. Journal of Applied Chemistry 11:435–443.

Dean, J.A. (ed.). 1978. *Lange's Handbook of Chemistry,* 12th ed. McGraw-Hill Inc., New York, New York.

Deer, W.A., R.A. Howie, and J. Zussman. 1966. *An Introduction to the Rock Forming Minerals*. Longmans, Green, and Co. Ltd., London, UK. 528 pp.

Down, J.L., M.A. MacDonald, J. Tétreault, and R.S. Williams. 1996. Adhesive testing at the Canadian Conservation Institute—An evaluation of selected poly(vinylacetate) and acrylic adhesives. Studies in Conservation 41(1):19–44.

Feigl, F. and V. Anger. 1972. *Spot Tests in Inorganic Analysis,* 6th English ed. (translated by R.E. Oesper). Elsevier Publishing Company, Amsterdam, Netherlands. 669 pp.

Ferraiolo, J.A. 1982. A systematic classification of non-silicate minerals. Bulletin of the American Museum of Natural History 172:(1)viii + 237 pp.

Ford, W.E. 1932. *Dana's Textbook of Mineralogy,* 4th ed. John Wiley and Sons, Inc., New York, New York. 851 pp.

Franey, J.P., G.W. Kammlott, and T.E. Graedel. 1985. The corrosion of silver by atmospheric sulfurous gases. Corrosion Science 25(2):133–143.

Graedel, T.E. 1984. Concentrations and metal interactions of atmosphere trace gases involved in cor-

rosion. *Proceedings, International Congress on Metallic Corrosion,* 9th International Congress, Toronto, Ontario. Pp. 396–401.

Green, L. 1989. A re-evaluation of lead conservation techniques at the British Museum. International Restorer Seminar, 1–10 July, Veszprém, Hungary. Pp. 121–130.

Grzywacz, C.M. 1995. Air Quality Monitoring. Pp. 197–209 in *Storage of Natural History Collections: A Preventive Conservation Approach* (C.L. Rose, C.A. Hawks, and H.H. Genoways, eds.). Society for the Preservation of Natural History Collections, Iowa City, Iowa. x + 448 pp.

Grzywacz, C.M. and N.H. Tennent. 1994. Pollution monitoring in storage and display cabinets: Carbonyl pollutant levels in relation to artifact deterioration. Pp. 164–170 in *Preventive Conservation: Practice, Theory and Research.* (Preprints of contributions to the Ottawa Congress) International Institute for Conservation, London, UK. 244 pp.

Haiad, J.C., J. Druzik, J.M. Ayres, and H. Lau. 1990. Museum environmental requirements: A literature survey. Transactions of the American Society of Heating Refrigeration and Air Conditioning Engineers 96(2)3391:112–121.

Hawks, C. and D. von Endt. 1990. Mercury and mercury compounds in natural history collections: An annotated biography. Natural History Conservation 5:4–19.

Hawthorne, F.C. 1983. The crystal chemistry of the amphiboles. Canadian Mineralogist 21(2):173–480.

Howie, F.M.P. 1984. Conservation and storage: Geological material. Pp. 308–322 in *Manual of Curatorship* (J.M.A. Thompson, ed.). Butterworths and the Museum's Association, London, England. 553 pp.

Michalski, S. 1994. Leakage prediction for buildings, cases, bags, and bottles. Studies in Conservation 39:169–186.

Miles, C.E. 1986. Wood coatings for display and storage cases. Studies in Conservation 31(3):114–124.

Nicholls, J.R. 1934. Deterioration of shells when stored in oak cabinets. Journal of Chemistry and Industry 53:1077–1078.

Padfield, T., D. Erhardt, and W. Hopwood. 1982. Trouble in store. Pp. 24–27 in *Science and Technology* (Preprints of contributions to the Washington Congress) International Institute for Conservation London, UK. 185 pp.

Palache, C., H. Berman, and C. Frondel. 1944. *The System of Mineralogy of James Dwight Dana and Edward Salisbury Dana,* Vol. 1, 7th ed. John Wiley and Sons, Inc., New York, New York. 1124 pp.

Palache, C., H. Berman, and C. Frondel. 1951. *The System of Mineralogy of James Dwight Dana and Edward Salisbury Dana,* Vol. 2, 7th ed. John Wiley and Sons, Inc., New York, New York. 834 pp.

Pearl, R.M. 1975. *Cleaning and Preserving Minerals,* 4th revised ed. Earth Sciences Publishing Company, Colorado Springs, Colorado. 86 pp.

Soto, L., J.P. Franey, T.E. Graedel, and G.W. Kammlott. 1982. On the corrosion resistance of certain ancient Chinese bronzes. Abstract of the Poster Sessions, International Institute for Conservation, 9th International Congress, Washington, DC. 23 pp.

Strunz, H. 1970. *Mineralogische Tabellen. Eine Klassifizierung der Mineralien auf Kristallchemischer Grundlage.* Akademische Verlagsgesellschft, Geest and Portig K.-G., Leipzig. 621 pp.

Tennent, N.H. and T. Baird. 1985. The deterioration of Mollusca collections: Identification of shell efflorescence. Studies in Conservation 30:73–85.

Tennent, N.H. and T. Baird. 1992. The identification of acetate efflorescence on bronze antiquities stored in wooden cabinets. The Conservator 16:39–47.

Tennent, N.H., J. Tate, and J. Cannon. 1993. The corrosion of lead artifacts in wooden storage cabinets. Scottish Society for Conservation and Restoration 4(1):8–11.

Tétreault, J. 1992. La mesure de l'acidité des produits volatils. Journal of the International Institute for Conservation—Canadian Group 17:17–25.

Tétreault, J. and E. Stamatopoulou. 1997. Determination of concentrations of acetic acid emitted from wood coatings in enclosures. Studies in Conservation 42(3):141–156.

Tétreault, J., J. Sirois, and E. Stamatopoulou. 1998. Studies of lead corrosion in acetic acid environments. Studies in Conservation 43(1):17–32.

Thomson, G. 1986. *The Museum Environment,* 2nd ed. Butterworth and Co. Ltd., London, England. 293 pp.

Waddington, J. and D.M. Rudkin. 1983. Relocation of the Royal Ontario Museum Paleontology Col-

lections, 1981. In *Proceedings of the 1981 Workshop on Care and Maintenance of Natural History Collections* (D.J. Faber, ed.). Syllogeous 44:93–101.

Waller, R.R. and D.E. McAllister. 1987. A spot test to distinguish formalin from alcohol solutions. Curator 30(3):240–249.

Waller, R.R. 1990. Pyrite oxidation at 25°C and 10 to 90 percent relative humidity. Program and Abstracts, Society for the Preservation of Natural History Collections, 5th Annual Meeting, 7–11 May, Chicago. Pp. 25–26.

Waller, R.R. 1992. Temperature and humidity sensitive mineralogical and petrological specimens. Pp. 25–50 in *Natural History Collections, I. Earth Sciences* (F. Howie, ed.). Butterworth Series on Conservation in the Arts, Archeology and Architecture, London, UK. xv + 138 pp.

Waller, R. 1999. Internal pollutants, risk assessment and conservation priorities. Preprints, International Council of Museums, Committee for Conservation, 12th Triennial Meeting, Lyon, France. Pp. 113–118.

APPENDIX 1—SUPPLIERS

Macherey-Nagel nonbleeding pH paper for the range 0–6, Quantofix® sulfite ion paper, and lead acetate paper are available from Aldert Chemicals Ltd., 648, Finch Avenue East, Willowdale, Ontario M2K 2E6, Canada.

Rhoplex N-560, a pressure sensitive acrylic adhesive, has undergone testing at the Canadian Conservation Institute (CCI) and was shown to have good ageing properties (Down et al. 1996 and Conservation Materials Database [MCIN] number 210). It is supplied by AACS International Guilders' Supplies, 12-1541 Startop Road, Ottawa, Ontario K1B 5P2, Canada.

Gas permeation tubes and wafer devices are manufactured and calibrated by VICI Metronics, 2991 Corvin Drive, Santa Clara, California 95051, USA.

Palladium chloride is manufactured by EM Science, a division of EM Industries Inc., Cherry Hill, New Jersey 08034, USA.

ARCHAEOLOGICAL ARTIFACT ATTRITION: TIME'S ARROW AND COLLECTION DEPLETION

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Abstract.—Archaeological collections from California containing human remains undergoing strict inventory and reconciliation protocols and representing 5 decades of accession were entered into a microcomputer-managed database and reconciled against the original excavation records. Artifact losses were tracked to determine the cause of collection attrition. Subsequently, various intuitively derived hypotheses regarding artifact loss were tested and found wanting. However, fresh hypotheses that more adequately explain artifact loss were next suggested and tested. The results are important to both archaeologists who have a professional stake in the intact preservation of entire collections and museum professionals concerned about the "critical paths" collections under their care, experience.

Sonderman (1996:28) makes the observation that archaeological collections differ from other museum collections, because archaeological objects, ". . . are not viewed as individual objects, not as tables or chairs might be, but rather, each flake or sherd is seen as part of the context from which it is recovered. It is not the individual object but the entire assemblage that is used to interpret the past." Trimble and Meyers (1991) provide a blueprint for the curation of intact archaeological collections by the strict enforcement of federal agency guidelines. Nevertheless, arguing that limited budgets, a downsized work force, restructuring, space limitations, and storage costs have impacted museums, Sonderman (1996) has also insisted upon an intellectually justifiable deaccessioning program designed to selectively cull archaeological materials from a glutted museum environment. This seeming contradiction is at the core of potential conflict between those who argue for thinning archaeological collections and those who defend their unaltered retention (McManamon 1996).

At the heart of the debate is the acknowledgment that archaeological collections are tools. This view argues that future archaeologists will "dig" museum collections as eagerly as they will sites. Today this is customary only when archaeological contexts are either exhausted or on the verge of becoming extinct such as with the case of San Francisco Bay shellmounds (*cf.* Broughton 1994, Lightfoot 1997, Simons 1992). Yet, when museum collections are used for research, it is essential that the inquiry begin with (1) an analysis of the methods used to recover the objects, (2) the research objectives that defined the sampling strategy, and (3) the history of the collections themselves. It is this third element that will be addressed below in pursuit of cooperative relations between the archaeologist and the museum professional.

It is a thesis of this paper that the assumption that archaeological collections are immune to change after their accession by a museum repository is illusory. Attrition of archaeological assemblages stored within professionally curated contexts is often a reality that imperils the archaeologist's understanding of the past as inevitably as development destroys sites. Fowler and Fowler (1995:133) state, "Various processes and procedures operate on collections in the museum setting, changing their value and representativeness: cataloguing procedures, records

maintenance, storage, movement, exhibition history, conservation history, deaccessioning (including trading, condemnation, or outright loss), facilities, overall management, . . ." Until museum professionals and archaeologists begin to examine critically the process of this post-field transformation, neither profession can presume that curation is a pristine, albeit final, stage in the sequence of activities associated with historic preservation. Nor, it must be stressed, should archaeologists consider that the sampling errors of their predecessors are the only impediments to the accurate interpretation of curated materials. Repository attrition may be another factor serving to introduce significant sampling error, particularly in the case of older collections or those that have been managed by quasi-professional museum personnel.

It is suggested that the analysis of repository attrition is comparable to the archaeological study of any other post-depositional phenomenon influencing artifact distribution such as the study of taphonomy or the effect of artifact looting. The common element is that they serve to provide a baseline against which an investigator can assess possible shortcomings biasing archaeological analysis and interpretation. In effect, such analyses tell us what has to be considered before interpretation can begin.

This paper will discuss the documentation of artifact attrition from a large number of well-documented collections that were curated, reconciled, and reinventoried at a large centralized repository in northern California. The opportunity to analyze patterns of attrition within these collections was afforded by the implementation of microcomputer database technology that helped accomplish this burdensome task. The resulting patterns of attrition have led to suggestions about ways that curators, repository managers, and ultimately archaeologists can foresee this loss, avoid it, or compensate for it in the case of older, often poorly documented collections.

THE DATA

In anticipation of a final resolution regarding the disposition of human remains along with the artifacts that accompanied them, the California Department of Parks and Recreation (DPR) authorized an inventory of all such materials under their control (Kautz 1988, Woodward and Evans 1992). To guarantee an inventory relatively free of historical connection with the collections, DPR hired the author as a consultant to bring their collections containing human remains together in one place, to repackage both the human bone and all other collection objects in a respectful and professional manner, and to reconcile the presence of all collection items against the original notes associated with their excavation. During this last phase, objects identified with the burials in any way were designated as "burial associated." Given the enormity of the task, it was necessary to use microcomputer database software to organize the original documentation for the collections (the record management aspect), the collections themselves, and information regarding their specific location within the facility, with each set of records comprising a separate database. In addition to the author, a crew of six assistants performed these tasks that included three archaeologists, two museum professionals, and one human osteologist. The project and the curated assemblages are currently housed at the California State Museum Resource Center located at 2505 Port Street, West Sacramento, CA 95691.

Work on these collections was conducted between 1987 and 1988 and resulted in the successful management of over 1,034,800 objects along with human remains. Management activities included the following tasks: (1) movement of all germane collections and their records from each of the California State Parks to a central repository in northern California, (2) reconciliation of each of these collections against their excavation records and each assemblage's site catalog, (3) standardization of catalog nomenclature to produce comparable databases, (4) repackaging of all human bone and all collection artifacts using museum-quality materials with appropriate identification and when necessary, the cleaning, stabilizing, and labeling of objects and osteological remains, (5) development of a durable and secure storage facility, (6) coordination of all activities with Native American, cooperating federal agency, and California Department of Parks and Recreation representatives, (7) database recordation of all inventory activities, and (8) production of interim reports and a final report outlining these activities.

ARTIFACT ATTRITION

During the phase of activities designed to account for the presence or absence of artifacts, it was noted that as each archaeological site's artifact catalog was reconciled against the remaining portion of the collection, patterns of loss were evident that at first glance appeared counterintuitive. As the principal person charged with the conduct of these activities, the author had brought to the task some assumptions regarding the nature of artifact loss. It should be noted that because this work involved so many large collections amassed over the span of 5 decades, was accumulated in so many ways (e.g., archaeological excavations, museum donations, individual item and collection accessions from various agencies, etc.), and stored under the supervision of so many people with varied backgrounds, the inventory staff expected some loss, perhaps even considerable loss. However, artifact loss was not deemed an analytical problem because, other than random loss, it was assumed that explanations for attrition of specific artifacts would be fairly obvious. For example, some of these intuitively "obvious" assumptions included the following:

- It was assumed that whole categories of artifacts might be lost from individual accessions because they had been loaned for analysis and never returned. It was also assumed that such transfers may have lacked documentation. Materials susceptible to such loss might have included such things as all obsidian tools or a whole artifact class such as all ground stone.
- No difference was anticipated in the loss of prehistoric as compared to historic artifacts.
- It was assumed that small items were more vulnerable to loss because they were more apt to "slip between the cracks."
- It was ventured that should the collections have been exposed to theft, the more precious or eye-catching items were more likely to be missing.
- Finally, it was assumed that the older the collection, the more often the collection would have been exposed to any and all types of loss.

However, given some of the initial qualitatively derived observations, and to provide a quick check of the accuracy of these assumptions against reality, each assumption was proposed as a formal hypothesis, accompanied by a separate test.

First, to test whether time sensitive and/or whole categories of artifacts were missing as a result of their having been loaned for study, artifact categories were designated as either "attractive" for outside analysis (projectile points, shell and bone beads, charcoal samples, bone awls, etc.), or "unattractive" (cobbles, quartz crystals, hammer stones, unknown lithics, etc.). The Chi-square test (χ^2) was used to test the discrepancy between observed and expected results. The null hypothesis, that the artifact's category (attractive or unattractive) cannot predict whether an artifact may be lost or retained was confirmed ($\chi^2 = 12.788$ at $\propto = >0.05$). Therefore, the assumed outcome—that the more "attractive" categories would be more often missing—was falsified.

The second hypothesis proposed that there should be no difference between the relative loss of artifacts from historic sites and those from prehistoric sites. Although there were far fewer historic sites within this sample of sites, there were significant differences noted between them. For example, prehistoric sites had suffered an average attrition rate of 9.5% of total catalog entries, whereas historic sites had suffered less than 2% loss. These results suggested that there might well be an important difference between losses experienced by historic versus prehistoric sites.

The third hypothesis concerns the size and/or weight of the item, proposing that the smaller and lighter items should be more vulnerable to loss. A simple review of some of the more obvious members of some easily managed categories suggests a very different pattern. For example, among the designated "light" items, the losses experienced are as follows: projectile points (9% missing), bone awls (6%), shell beads (4%), shell or bone ornaments (7%), scrapers (7%), bone tools (5%), and flakes (6%). Compare these results to losses experienced by the heavier artifacts: manos (23% missing), pestles (26%), metates (32%), mortars (37%), "rocks" (29%), cobbles (29%), and soil samples (63%). It is possible, of course, that some of the soil samples had been consumed by using them to obtain soil chemistry data, however, the prediction regarding size/weight as a predictor of attrition was found to be false. Of more interest was the apparent relationship suggesting an opposite conclusion.

The fourth hypothesis suggests that the more showy and precious an item, the more it should have been exposed to theft (*cf.* Stothers and Abel 1989). To test the hypothesis, another set of two categories was created. The first category was composed of so-called "valuable" items made up of projectile points, musical instruments, pendants, knives, and charmstones. These were contrasted with items considered "less desirable." Items in the latter category included quartz crystals, cobbles, soil samples, and baked clay samples. Again, it was determined that it was far more likely that a "less desirable" item would be missing than a "desirable" one. An average of 4% of all the "desirable" items was missing, whereas an average of 21% of the "less desirable" items was lost. Again, the fourth assumption was proved wrong.

Finally, it was assumed that the longer a collection had been accessioned within the DPR system, the more items would be missing. This assumption was based upon the simple likelihood that more time provides more opportunity for loss. To test the hypothesis, the sites were grouped into decades (1940–1949, 1950–1959, etc.). For all catalog entries, it was found that 11.5% of the items are missing. The outcome of this analysis suggested that for each decade, the average loss

Table 1. Rank of artifacts ordered by weight and sequence of attrition.

Rank	Order by weight	Order by attrition
1	mortar	soil sample
2	metate	mortar
3	soil sample	metate
4	mano	mano
5	charmstone	charmstone
6	scraper	quartz crystal
7	projectile point	projectile point
8	quartz crystal	scraper
9	bead	bead

falls within several percentage points of the attrition mean for all the collections. This inevitably leads to the conclusion that the variation in attrition does not appear to be linked in any simple way to the age of the collection.

After having rejected all five of our initial assumptions/conjectures to explain the loss of artifacts, it was apparent that we knew next to nothing about how and why artifacts had been lost. As part of the overall task of protecting these collections we now began to examine specific variables that might better explain artifact attrition.

One quite important variable appeared to be size/weight. Although our initial prediction regarding size/weight proved wrong, its opposite appeared to render a far better forecast of artifact loss. In other words, the heavier/larger the object, the more likely it was that the object would be lost. This was tested using the Spearman rank correlation coefficient (r_s). The average weight of nine different items was determined and their average weights ranked. Then the same items were ordered according to their likelihood of being lost (Table 1). The results of the test provide a result of $r_s = 0.883$, or a significant correlation at the $P = 0.01$ level.

To understand better how increased weight may have increased the possibility of artifact loss, it was only necessary to recall what it was like to have relocated a number of these collections. Several of the mortars and metates weighed in excess of 170 pounds, and the decision to move such large items entailed the organization of people and equipment that may not always have been easily available, particularly at professionally understaffed state parks. Likewise, a common packaging behavior with many of the earlier collections was to pack all the ground stone artifacts together. When a quantity of the manos and pestles (ground stone) had been crated collectively, the resulting box unit was excessively weighty. This undoubtedly resulted in the temptation to put off moving the collection's heavier fraction. At this point it seemed sensible to combine several of the hypotheses offered above into a newly rendered premise: the older the collection, the more likely that the heavier objects will be missing. The reasoning being that the older collections would have endured more moves wherein more of the collection's more massive items would have become separated.

To test this new hypothesis, the collections were divided once more into two categories, representing "early" procedures and those representing more "modern" procedures. The arbitrary date selected was to distinguish between those

items excavated before 1965 and those excavated after 1965. Both early and more modern categories contained a significant number of assemblages and artifacts. Then, using the object categories of metate, mano, pestle, and cobble to represent the "heavy" objects from each collection, contrasted with the remainder of the collection, the following results were produced. In the "early" accessioned sites, the "heavy" object loss ranged between 18 and 58%, with an average of 27%. In those sites that were accessioned at a "later" date, the range of "heavy" object loss was between 0 and 25%, with an average of 8%.

A second postulate is considerably harder to describe. When an artifact category was found to contain items that were vaguely defined, were natural as opposed to cultural in origin, or with contents that were ambiguous in form such as "soil sample," "baked clay sample," "shell sample," or "feature soil," they appeared particularly vulnerable to loss. So too were objects that had experienced very little, if any, human modification such as "cobble," "rock," "pigment," "fire-fractured rock," "quartz crystal," or "unmodified lithics." From this observation it was inferred that at various times, these collections, in their original intact state, had experienced a decision process whereby some items had been discarded, while others had been retained.

It was further inferred that the value of an item to the collection had been judged according to its perceived relevance to "things archaeological" with a bias against those items only vaguely esteemed. What should have been considered, as most field archaeologists appreciate, is that items of this nature are retained for quite specific purposes. In fact, the precise reason these objects are retained is usually for future analysis at a time when more information regarding their relevance to the assemblage, the site, and the larger issues of theoretical archaeology, might be more fruitfully assessed.

Though this hypothesis regarding the possible effect of collection decision making is all very plausible in theory, it still remained to test it. Actually, it isn't particularly meaningful that "vagueness" constitutes a factor in artifact attrition, but rather, that the attrition is the result of a conscious decision made by a person who had the power to implement that decision. This element of a conscious manipulation is profoundly important because should it prove true, then nonprofessional staff responsible for collections can be educated to the practical difficulties introduced when they prune archaeological assemblages without benefit of formal deaccessioning procedures.

To test the above hypothesis and its ancillary inference, a questionnaire was distributed to ten museum professionals (not archaeologists) who agreed to cooperate anonymously without knowing the reason. The questionnaire required each to provide a ranking of items from a list of ten archaeological objects. It asked which items they would choose to cull if they were told to do so by museum management. The objects were naively defined as: "shell bead," "arrowhead," "scraper," "pigment," "soil sample," "quartz crystal," "stone core," "bone tool," "river cobble," and "knife." The next task was to average the results of the respondents, rank them, and compare that ranked order with a ranked order representing the frequency of loss for those same items. The Spearman rank correlation coefficient (r_s) was again used to compare the resulting rankings (Table 2).

The results ($r_s = 0.891$ with a $\propto = 0.01$) indicate a very strong correlation between a preference for archaeological item discard and the effect of that ap-

Table 2. Rank of artifacts ordered by selection for being discarded and sequence of attrition.

Rank	Order by selection	Order by attrition
1	river cobble	soil sample
2	soil sample	river cobble
3	stone core	stone core
4	pigment	pigment
5	quartz crystal	quartz crystal
6	scraper	knife
7	knife	bone tool
8	bone tool	arrowhead
9	shell bead	scraper
9	arrowhead	shell bead

parent behavior on the assemblages (i.e., agreement as to what should be discarded). The results also indicate a surprisingly similar consensus regarding the relative importance of archaeological items to the total assemblage as that value is expressed by those persons tested (i.e., agreement as to what should be retained). In other words, it seems to indicate an agreement with the hypothesis, that the more familiar an item is in terms of its morphology and use (bead, projectile point, etc.), the more likely the item will be valued, and therefore, the more likely it will be retained. This result may also help answer why historic items were less likely to be discarded. With a value hierarchy so strongly influenced by the evident familiarity of an item to the decision maker, even an unrecognizable puddle of metallic or glass slag would provide some measure of familiarity. It was apparently this familiarity that has served to protect more recent and familiar items from being discarded.

THE LESSONS LEARNED

The principal lesson from this exercise for both the archaeologist and the museum specialist is that curation is not a passive activity. The current condition of most museum collections is not the product of random forces acting independently or at odds with preservation-minded professionals. Rather, a collection's integrity may be the product of the values implemented informally by workers throughout the analytical and museum life of those collections.

Each time the museum staff prepares to move a collection, plans for a reorganization of space, or prepares an archaeological exhibit, archaeological materials may be affected. Therefore, museum staff must be reminded of their ethical and legal responsibility to maintain archaeological collections intact. This may force museum staff to spend more effort and expense during planning phases and more time with the training of ancillary personnel, particularly those at satellite facilities. As an example, it was found that collections that were curated away from the central DPR museum repository (i.e., those within the aegis of the separate state parks) had experienced far more patterned loss than those managed by the State of California's professional museum personnel. Analysis of the collections from the satellite state parks revealed that problems with their collections were exclusive to several of the parks but by no means to them all. Because this was the case, it was recommended that collections management and systematic oversight should continue to be exercised by museum professionals.

Archaeologists must take responsibility for the ongoing care of their collections. In an institutional setting, they must also preserve the collections resulting from the work of their predecessors. Curation agreements can be made to contain a requirement that the excavator or the agency for which the work was performed be informed before any formal or informal deaccession of excavated archaeological materials. It was our experience that where ownership and management status of the collection was most ambiguous, the collection was most at risk.

It is important to note the opportunity that projects such as the State of California, Department of Parks and Recreation's Burial Inventory provide for the research archaeologist. After the inclusion of information regarding the content of excavations at over 60 major archaeological sites and the standardization of the language used to describe the artifacts and artifact classes into an easily managed database, the opportunity to ask pertinent research questions was unparalleled. For example, it was determined that the assignment by the excavator of a burial's gender, often was dependent far more on the classes of burial furniture accompanying the burial, than on recognized morphological and osteometric indicators. This kind of circular reasoning (ground stone indicates a female's burial, hunting gear indicates a male's burial) has resulted in a number of erroneous conclusions and the mistaken assumption that the correlation is always accurate (*cf.* Gero 1991).

Although it is acknowledged that problems exist with using previously excavated museum materials from different excavations (incomparability of excavation procedures, differing sampling strategies, different research priorities, different post-excavation histories, etc.), it is recommended that archaeologists use curated collections more often and more intelligently. It is undoubtedly true that the manpower required to reexamine and standardize existing museum collections for comparative purposes is far more cost effective and time effective and results in far less site destruction than its analytical alternative; the implementation of a regional research design. Nevertheless, during the 2 yr that this immense corpus of data was being processed by the Burial Inventory staff, only one application was received from an archaeologist requesting collection access, and that scholar wished access to the content of only a single collection. This transpired despite the author having contacted nearby educational institutions possessing graduate training programs and offered to assist either teaching staff or their graduate students with their research needs. The only complicating factor was administrative, that of obtaining authorization for access to the collections (Native American, Director of DPR, etc.).

Finally, it is my belief that the time to cull archaeological collections is before their being accessioned. This is the only moment when the principal archaeologist (excavator and assemblage analyst) has enough information to render a rationale for discard and retention. That rationale should be explicitly stated in the text of the site report, in the site catalog, and in the letter of transmittal accompanying the collection. If the lead agency, the museum, or a review agency such as a state historic preservation office questions that rationale, then the items slated for disposal can be reintroduced into the collection. Once this moment is past, the maintenance of the integrity of the archaeological collection is a legal and ethical matter entirely in the hands of the curatorial facility's professional museum staff under direction of that assemblage's curation agreement.

LITERATURE CITED

Broughton, J.M. 1994. Declines in mammalian foraging efficiency during the Late Holocene, San Francisco Bay, California. Journal of Anthropological Archaeology 13:371–401.

Fowler, C.S. and D.D. Fowler. 1995. Formation processes of ethnographic collections: Examples from the Great Basin of western North America. Pp. 129–144 in *Learning from Things: Method and Theory of Material Culture Studies* (D. Kingery, ed.). Smithsonian Institution Press, Washington, DC. 262 pp.

Gero, J.M. 1991. Genderlithics: Women's roles in stone tool production. Pp. 163–193 in *Engendering Archaeology: Women and Prehistory* (J.M. Gero and M.W. Conkey, eds.). Basil Blackwell, Oxford, England. 418 pp.

Kautz, R.R. 1988. Final report of the Burial Inventory Project. Manuscript on file with the Office of Interpretive Services, California Department of Parks and Recreation, West Sacramento, California. 40 pp.

Lightfoot, K.G. 1997. Cultural construction of coastal landscapes: A middle Holocene perspective from San Francisco Bay. Pp. 129–141 in *Archaeology of the California Coast During the Middle Holocene* (J.M. Erlandson and M.A. Glassow, eds.). Vol. 4, Institute of Archaeology, University of California, Los Angeles, California. 187 pp.

McManamon, F.R. 1996. In context: The long view. Common Ground 1(2):2.

Simons, D.D. 1992. Prehistoric mammal exploitation in the San Francisco Bay area. Pp. 73–104 in *Essays on the Prehistory of Maritime California* (T.L. Jones, ed.). Publication 10, Center for Archaeological Research at Davis, Davis, California. 277 pp.

Sonderman, R.C. 1996. Primal fear: Deaccessioning collections. Common Ground 1(2):27–29.

Stothers, D.M. and T.J. Abel. 1989. A tabulation and analysis of stolen antiquities from the historic Ottawa Cabin at the Fry Site. Ohio Archaeologist 1:26–37.

Trimble, M.K. and T.B. Meyers. 1991. *Saving the Past from the Future: Archaeological Curation in the St. Louis District.* Department of the Army, Corps of Engineers, St. Louis District, Illinois. 149 pp.

Woodward, J. and N.H. Evans. 1992. Summaries of burial collections. Manuscript on file with the Cultural Heritage Section, California Department of Parks and Recreation, Sacramento, California. 206 pp.

DIFFERENT DEGRADATION EFFECTS ON MIOCENE WHALE SKELETAL REMAINS FROM GRAM, DENMARK, CAUSED BY THE CLAY MATRIX

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Abstract.—Two pyritiferous whale bone skeletal remains obtained from Miocene clay (Gram Formation) were found to be in different states of preservation, although they had been deposited in more or less the same environment and stored under identical conditions. One of the skeletons was embedded primarily in light-coloured clay dominated by the mineral smectite with a high absorption capability and low pH. The other embedding medium consists of other clay minerals, mainly kaolinite and illite, having a neutral pH that can be explained by the presence of carbonates, which work as buffers. Pyritiferous fossil bones from smectite-rich clays appear to be more susceptible to deterioration after exposure than those preserved in clays dominated by other clay minerals.

During excavations of palaeontological material, differences in the state of preservation of fossils found in the same formation have often been observed. Previous studies, such as those by Aaris-Sørensen (1978), Lyman (1994), and Child (1995) have shown that bones buried at the same time, though excavated on the same occasion, may demonstrate considerable differences in the state of preservation. Such differences have been noted even between bone specimens deposited just a few centimetres apart. Berggren et al. (1995) clearly illustrate two fossil whales (*Balaenoptera* sp.) that, in spite of sharing the same history of being embedded in the sediment, show considerable differences in degradation. According to these authors, a plausible reason for the differences observed may be a synergetic interaction between bone histology, clay content and structure, and factors such as relative humidity (RH) and pH.

The whale bone locality is a clay pit situated 1 km north of Gram, in southwestern Jutland, Denmark (Fig. 1). The Gram Formation is represented by some 20–30 m of shallow marine clays originating from a transgression during the Late Miocene (Beyer 1987, Rasmussen and Larsen 1989).

In 1982, when the clay was commercially exploited, three fossil whale skeletons were discovered. So as not to hinder the clay mining, the fossil whales had to be removed in a hurry. Large blocks of clay (each ca. 200 kg, 170 × 80 × 60 cm) containing the whale skeletons were removed from the pit. Each block was then wrapped with polyethylene. In 1993, two of the fossil whales in question needed to be prepared for an exhibition (skeletons P.818 and P.819). Shortly after the removal of the polyethylene and some of the clay matrix (the clay that adhered to and surrounded the bones), one of the whales (P.818) started to degrade. In some areas the clay was damp, but dried out very quickly. Cracks in the bone, signs of yellow and white weathering products on the surfaces of the bones, and the smell of sulphur led to the first impression that this chemical breakdown was linked mainly to pyrite content accompanied by the weathering product, sulphuric acid.

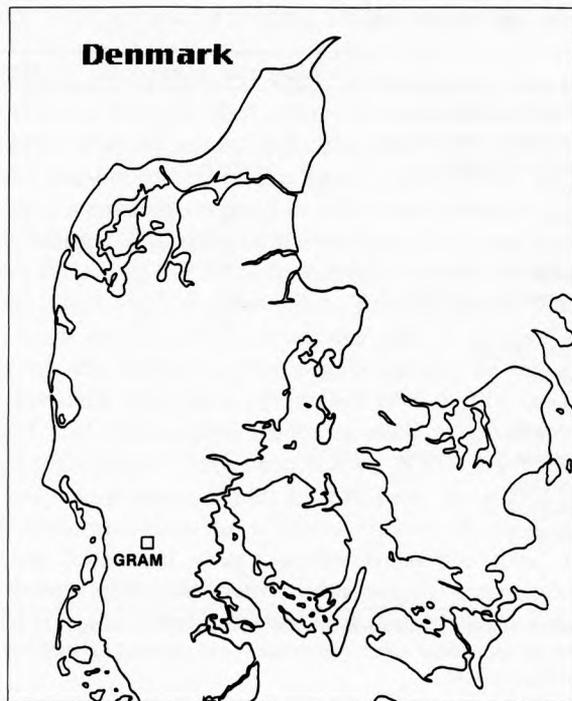It was of major importance to find out why one of the whale specimens, but

Figure 1.   Location map of Gram, south-western Jutland, Denmark.

not the other, started to break down, because both of them (P.818 and P.819) originated from the same site and had been stored for 12 yr under identical conditions. The problem justified an investigation, which started at Gram in 1994. That project included three main parts: (1) an analysis of the histology of the fossil whale bones and description of the differences, both histological and preservational, in skeletons P.818 and P.819, (2) an analysis of the clay matrix that adhered to these bones, and (3) identification and characterisation of the weathering products visible on the bones and clay. The results of the first part of the investigation are reported in Berggren et al. (1995). In the present paper, the focus is on the second part, the influence that the clay matrix had on the preservation or degradation of the whale bones.

Visual differences, resulting from the differing colour and structure of the dry clays coating the two whale bone skeletons, were found. Skeleton P.818 was surrounded mainly by light-coloured porous clay, whereas P.819 was mostly coated by darker, more compact clay.

METHODS

For the present study, analyses were carried out on clays that differed in colour and structure and that coated the degraded as well as nondegraded bones. Samples of both types of clay taken at some distance from the fossil bones were also analyzed. For the study of the clays the following equipment and tests were used: (1) scanning electron microscope/energy dispersive analysis x-ray (SEM/EDAX) was used for element identification (although F, O, and C were not detectable by

Table 1. Content of the light and dark clays.

		Light clay (porous)	Dark clay (compact)
Clay mineral	Tosudite (smectite)	X	—
	Nontronite (smectite)	X	—
	Smectite-kaolinite	—	X
	Illite	—	X
Silicates	Quartz	X	X
	Muscovite	X	X
	Lepidolite (mica)	X	—
	Aluminium silicate	X	—
Iron sulphides	Pyrite	X[a]	X[b]
	Chalcopyrite	—	X
Carbonate	Calcite	—	X
	Siderite	—	X
	Dolomite	—	X
Sulphates	Gypsum	X[c]	X
	Alunogen	X	—
Phosphates	Phosphates	X	X
Trace matter	Titanium	X	X
pH		3.5–4.0	6.5–7.0
Porosity		pore volume 0.143 cm³/g	pore volume 0.115 cm³/g

[a] The pyrite is spread in single framboides, which are partially oxidised.
[b] The pyrite is spread in aggregates (poly framboides) and in single framboides.
[c] Found as a weathering product.

this equipment), (2) x-ray fluorescence (XRF) was used for quantitative element identification (distinguishing between F and U), (3) x-ray diffraction (XRD) (Siemens diffraktometer D 5000) was used to identify minerals according to the Siemens kristalloflex scale, (4) mercury porosimetry was measured using a Micromeritics pore sizer 9305 to determine pore volume and pore size, and a (5) pH-test was completed.

Because the differences in degradation of the bone were so clearly correlated to the colour and structure of the clay, the analysis was based on these differences. The light and the dark clays are located in well-defined areas (ranging in size from centimetres to decimetres) of both the specimens.

RESULTS

The analytical methods demonstrated the different mineral compositions in the clay of the two specimens. Some similarities and differences between the light, porous clay and the dark, compact clay are shown in Table 1. Using several analytical methods, differences in the mineralogical and elemental composition of the two clay types were found.

Chemical Composition, Acidity, and Porosity of the Light and Dark Clays

For the light clay, XRD analysis showed clay minerals belonging to the smectite group, which are rich in magnesium. A variety of aluminium silicates were also identified. SEM-EDAX examination for element identification and microscopic examination showed the presence of elements such as iron (Fe), sulphur (S), and copper (Cu). The clearly visible crystals on the surface of the dried blocks of

clay were gypsum. Acidity measurements of the light clay gave pH values of 3.5–4.0.

Analysis of the dark clay produced some similarities to the light clay, as well as differences, in mineral and element composition. In addition to smectite, XRD analysis detected other clay minerals, including illite and kaolinite. Sulphides such as chalcopyrite (not present in any of the light clay samples) and pyrite were also identified. Pyrite is a common mineral in clay and often forms under reducing environmental conditions. The type of pyrite found is mostly framboidal, which is microcrystalline. By using the SEM technique, the framboidal aggregates were clearly visible. The XRD analysis identified mineral groups that were not shown in the light clay, such as different carbonates and sulphates. Gypsum was not visible as crystals on the surface of the bigger blocks of the dark clay. Acidity measurements of the dark clay gave pH values of 6.5–7.0.

In both the light and dark clays, minerals such as quartz, muscovite, and a variety of types of phosphates were detected by XRD. By using XRF for quantitative element analysis, potassium and magnesium, were also detected. Samples taken at different distances from the whale skeletons showed that the amount of phosphorus detected decreased with distance from the bone. Phosphorus is an element that can be clearly connected to the bone mineral apatite. Analyses of pore volume and size in the clays indicated that the light clay has a higher porosity than the dark clay (Table 1).

DISCUSSION

Although the similarities in the light and dark clays were considerable, the differences are more interesting, because the need was to find out if, and how, the clays had affected the bones in different ways. Looking at the clay minerals revealed that the light clay was dominated by smectite. When drying, the smectite shrinks and cracks. The dark clay consists not only of smectite, but also of illite and kaolinite, which have much less tendency to crack and shrink during the drying process (Deer et al. 1992, Grim 1968, Krauskopf 1979). The drying processes have different effects on the degradation of the whale bones. When the light clay cracks into smaller parts its support for the skeletons disappears. This allows oxygen to come into contact with the bones and with pyrite in the sediment. There may also be a mechanical effect of the forces connected to the drying process. The dark clay dries in bigger lumps and retains its contact with the bones, which probably protects them to some extent. The higher porosity of the light clay may also have an effect on the drying process.

For the light clay, the detection of the elements S and Fe and also a few partially oxidised single pyrite framboids, may show that an oxidation of pyrite in the light clay has occurred. A factor that supports this theory is the low pH of the light clay. Decay products have also been found on the surface in the form of gypsum and sulphur. This can also be attributed to the light clay's tendency to crack more, because the pyrite in the light clay probably oxidised when the proportion of oxygen increased in connection with crack formation during drying of the clay. The pyrite that then oxidised to sulphuric acid could react with the clay. This may have caused even further oxidation.

For the dark clay none of the above-described processes has occurred. The analyses showed that the clay does contain pyrite. It also contains carbonates,

which can be neutralising the sulphuric acid in case of oxidation of the pyrite. The neutral pH observed supports this theory. The pore volume may also explain the tendency for a higher exchange of ions between the bone and the clay that has taken place in the light clay. Finally, note that the degraded skeleton was embedded in light clay and the nondegraded skeleton in dark clay.

CONCLUSIONS

Major differences in the degradation process have been observed in fossil objects deposited only a few centimetres apart. Some of the reasons why one clay may have a stronger degrading effect on fossil bones than another clay seem to be the following:

- The light-coloured clay was rich in smectite, which would contribute to the tendency to swell in wet or shrink in dry conditions. Cracks from shrinkage would favour oxidation by exposing more surface area to the air.
- The amount of pores and the pore volume are also significant factors. The larger and more frequent the pores, the higher the risk of oxygen influence. When pyrite oxidises, lighter clays tend to be degraded more easily by sulphuric acid than do darker clays. One of the reasons may be the lack of a carbonate buffer in the light clay.

The matrix was exposed to uncontrolled drying during a period of 12 yr. This leads one to conclude that some of the observations can be confirmed only after comparable study of newly excavated material. The extent of shrinkage, the acid resistance, and the effect of organic material on the clay need to be further analysed.

ACKNOWLEDGMENTS

LITERATURE CITED

Aaris-Sørensen, K. 1978. *Knoglematerialet fra den mellemneolitiske boplads ved Korsnäs.* Riksantik-varieämbetet och Statens historiska museer, 1978:8, Stockholm. 28 pp.

Berggren, K., I. Chef-Johansson, and I. Vestergaard. 1995. *Konserverings-problematik kring miocena valskelett, Gram, Danmark.* Institute of Conservation, Göteborg University. 84 pp.

Beyer, L. 1987. *En palaeomagnetisk undersögelse av Gram Formationen.* Midtsønderjyllands Museum Slot, Gram. 48 pp.

Child, A.M. 1995. Microbial taphonomy of archaeological bone. Studies in Conservation 40(1):19–30.

Deer, W.A., R.A. Howie, and J. Zussman. 1992. *An Introduction to the Rock-Forming Minerals,* 2nd ed. Longman Scientific and Technical Publications, New York, New York. 696 pp.

Grim, R.E. 1968. *Clay Mineralogy,* 2nd ed. McGraw-Hill Book Company, New York, New York. 596 pp.

Krauskopf, K.B. 1979. *Introduction to Geochemistry,* 2nd ed. McGraw-Hill, New York, New York. 617 pp.

Lyman, R.L. 1994. *Vertebrate Taphonomy.* Cambridge Manuals in Archaeology, Cambridge University Press, Cambridge. 524 pp.

Rasmussen, E.S. and O.H. Larsen. 1989. *Mineralogi og geokemi af det Øvre Miocæne Gram ler.* Danmarks Geologiske Undersøgelse, serie D 7, København, 81 pp.

THE WARPING AND CRACKING OF PLEXIGLAS™ SPECIMEN CONTAINERS

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Abstract.—In the 1960s Plexiglas™ specimen containers were introduced in the anatomical collection of the Leiden Medical Faculty and in time replaced the cast rectangular glass jars. It was assumed that the Plexiglas™ jars would be less fragile and therefore better suited for use in medical teaching. Recently, we noticed that Plexiglas™ jars, after being filled and sealed, gradually warp to the inside and finally crack at the glued joints. We studied three experimental models to determine that the causes for the warping and cracking of Plexiglas™ jars are the result of the absorption of water by the Plexiglas™ and its slow diffusion through the acrylic.

For more than 300 yr, fluid-preserved specimens have been an important tool in medical teaching. For demonstration purposes, it is essential that the specimens are stored in clear transparent containers. Until the 1930s, glass was the only suitable material. Initially, only cylindrical shaped jars were used, but by the end of the 19th century, rectangular-shaped jars were also available. The great advantage of these jars is their lack of visual distortion. However, because of the fragility of glass, the jars were kept in cabinets and rarely allowed in the hands of students.

In the early 1930s, polymethylmetacrylate (PMMA) was introduced under the names Perspex™ and Plexiglas™. This new synthetic material was, like glass, clear, transparent, and highly water resistant. The cast sheet material can be cut to a preferred size and shaped by thermoforming. Sheets can be glued together with chloroform or a polymer/monomer slurry (PMMA/MMA).

In the Leiden Anatomy Museum, Plexiglas™ jars were introduced in the collections in the 1960s. They had a great advantage for medical teaching compared to glass containers. Because of its flexibility, PMMA seemed to be less vulnerable to cracking and therefore safer to handle. We discovered that, in time, there were also disadvantages in the use of acrylic jars (van Dam 2000). It appeared that the Plexiglas™ jars, which were filled with either 10% formalin (3.6% formaldehyde in water) or 75% glycerin (64% glycerol in water), gradually warped inward after being filled and sealed (Fig. 1). Although for periods of 10 yr or more the containers showed no visual decline in fluid level, the level dropped significantly (5–10%) when the container was aerated. The glued joints showed little cracks from the outside inward (Fig. 2). These observations indicated that the sides of the jars had been under considerable stress. Consequently, upon inspection, several jars were found leaking and a few jars spontaneously burst at the joints. Apparently, when the jars are aerated, the observed fluid loss is not compensated for by air intake, and consequently a negative pressure is created inside the jar (van Dam 2000). This causes the jar to warp to the inside and the glued connections to
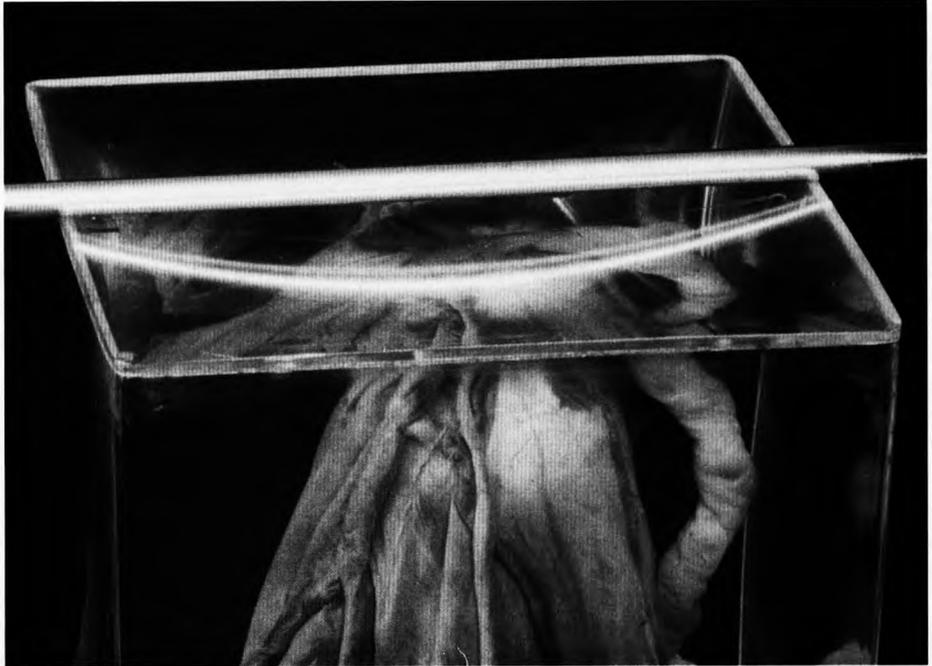
Figure 1. Warped Plexiglas™ jar. The reflection of the metal rod shows the extent of warping of the upper side.

Figure 2. Partially split seam of a Plexiglas™ jar.

weaken from stress cracks. In this fragile condition these specimen jars are hardly suitable for medical teaching.

At present, specimens in leaky Plexiglas® containers, and newly prepared specimens, are placed in cast rectangular glass jars. However, these are difficult to find now, and are available only in a few sizes. For these reasons, there is an urgent need to determine the causes for the warping and cracking of Plexiglas® to find solutions to these problems. In this paper, several experimental models were used to study the mechanism of the warping and cracking of fluid filled, sealed Plexiglas® containers. The causes and possible solutions for these problems are discussed in the context of the experimental results.

MATERIALS AND METHODS

Three experiments were carried out to determine whether the observed fluid loss, negative pressure, and warping of the jars could be related to the permeability and absorption properties of Plexiglas®.

Absorption experiment.—The purpose of this experiment was to determine which components of the fluid are absorbed most readily by the Plexiglas®. Small Plexiglas® sheets (25 × 30 × 6 mm) were placed in demineralized water, glycerol, and formaldehyde solutions for 50 days. Immediately before and after the experiment the sheets were weighed. The next two experiments were based on the outcome of the absorption experiment.

Pressure experiment.—The purpose of this experiment was to determine if, in a hermetically sealed Plexiglas® jar, fluid loss by diffusion could be related to the observed negative pressure in the Plexiglas® jars in the museum. An 8 mm thick cylindrical Plexiglas® container with a volume of 560 ml was totally filled with demineralized water, excluding as much air as possible. Pressure and temperature inside the jar were monitored by sensors and recorded by means of a computer, so that the jar could be disconnected during the experiment for weighing (Fig. 3).

Warping experiment.—The purpose of this experiment was to determine if there would be a difference in the extent of warping in closed and open (vented) systems. Two cylindrical glass containers, each with a diameter of 35 cm and volume of 10 L were filled with demineralized water and closed with a 2-mm thick Plexiglas® lid with a 6-mm drilled hole approximately 2 cm away from the outer edge. The lids were sealed with bitumen (Shell Tixophalte®). Both jars were topped up with water to a level of 1.5 cm below the rim, and the holes in the lids were left open. One day later, one of the jars was totally filled, including some small air pockets underneath the lid. The hole in the lid was closed with a small sheet of 2-mm thick Plexiglas®, which was sealed with Acrifix 190 to make the closed system. The vertical displacement of the center of the lids was monitored by a micrometer with its sensor tip placed in the center, on top of the lid. A temperature probe was placed close to the jars to record ambient temperature (Fig. 4).

RESULTS

Absorption experiment.—Table 1 shows the weight change of the Plexiglas® samples after having been soaked in water and different concentrations of glycerin and formalin. The results of this experiment show that the higher the water content

Figure 3.   Experimental setup to measure the pressure drop inside a water-filled, sealed Plexiglas®
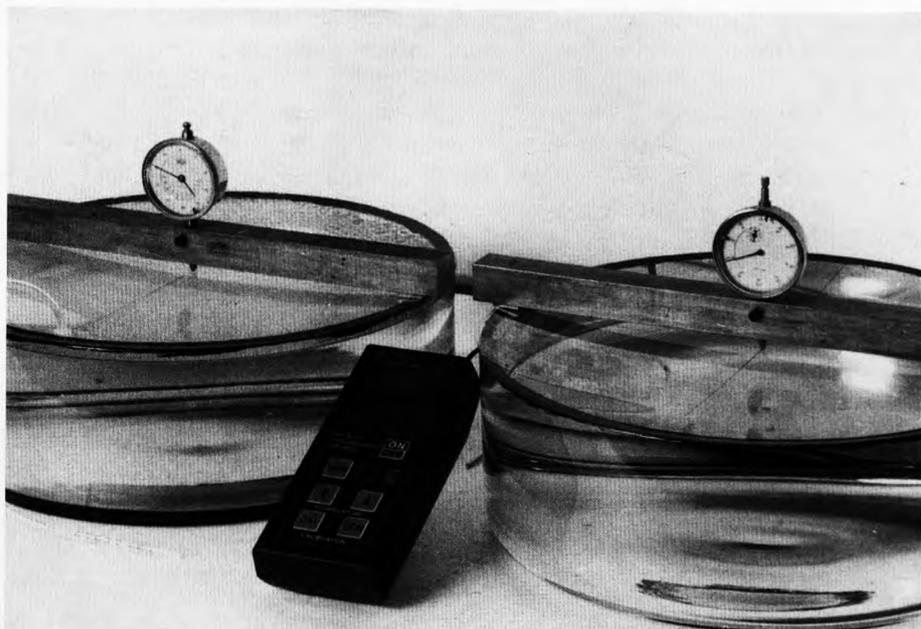container.

Figure 4.   Experimental setup to measure the inside warping of Plexiglas® in open and closed con-
tainers filled with water.

Table 1. Weight increase of Plexiglas® samples after immersion in water, formalin, and glycerin for a period of 50 days.

Fluid	Concentration (% w/w)	Weight increase (%)
Formaldehyde in water	3.3	0.707
	9	0.644
	18	0.558
	Concentration (% v/v)	
Glycerol in water	20	0.662
	30	0.539
	45	0.428
	67	0.204
	100	−0.394
Water	100	0.750

in the mixture, the greater the weight increase of the sample. The greatest increase can be seen in the sample soaked in pure water. A decrease in weight was recorded for the sample soaked in 100% glycerol.

Pressure experiment.—The pressure/time graph for the water-filled, sealed Plexiglas® cylinder (Fig. 5) shows the decrease in internal pressure during a time period of approximately 200 days measured at an ambient temperature of 22.0 ± 0.5°C. The pressure readings on the internal probe dropped in the first month to −0.1 atm (−103 g/cm²). From this point, it took another 4 mo to drop to a reading of −0.2 atm (−207 g/cm²). The graph shows a linear regressive trend in the first 30 days followed by a less regressive trend from day 50 onward. The weight readings recorded a loss of 1.5 g at day 110 and 1.7 g at day 204.

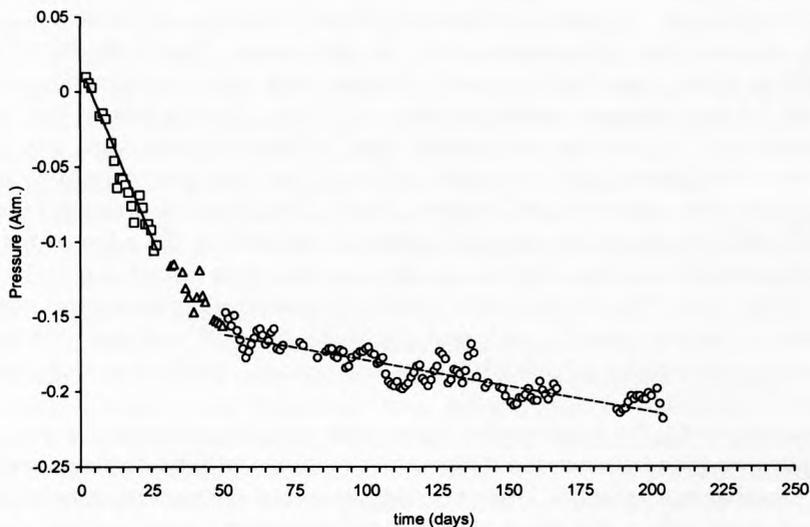*Warping experiment.*—The graph for the warping of the Plexiglas® lids (Fig.

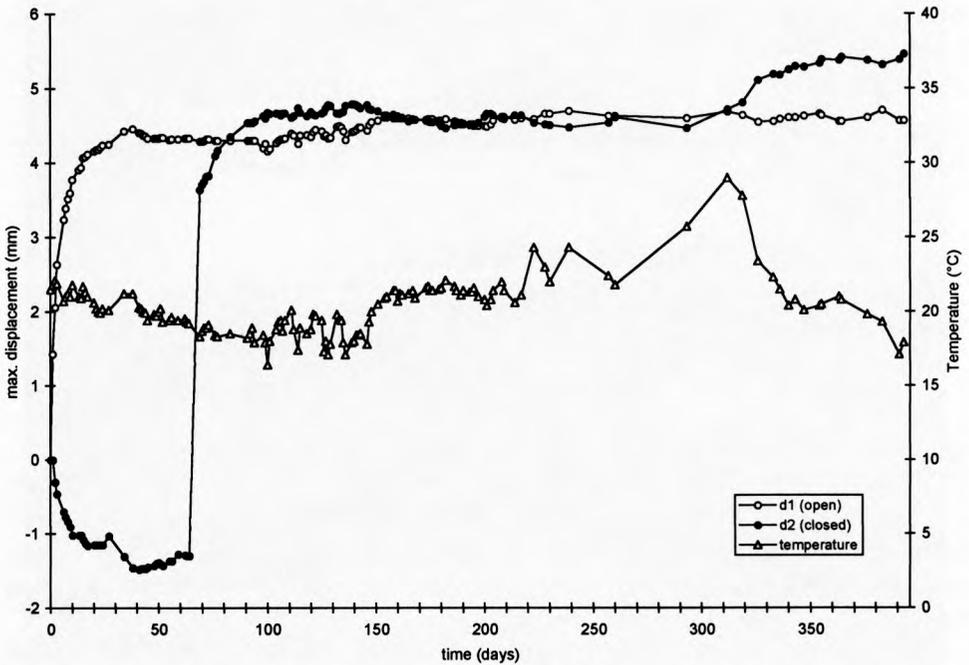Figure 5.   Pressure/time graph for the water-filled, sealed Plexiglas® vessel.

Figure 6.   Displacement graph for the center of the Plexiglas^{TM} lids in open and closed glass containers.

6) gives the daily measurements of the displacement of the center of the Plexiglas® lids and the ambient temperature. The readings for the open system show, in the first 3 days, a rapid downward displacement reaching a value 2.64 mm. During the next 30 days the curve gradually levels off around 4.4 mm.

The graph for the closed system has its starting point at the reading of day 1. This first reading of −1.64 mm (downward displacement) is the result for an open (vented) system. From this point on the jar was closed. During the first 34 days the readings show a gradually upward displacement and a stabilization between day 34 to 64 at a value of approximately −1.3 mm. During this 64-day period, the formation of air bubbles underneath the lid was noticed. From day 1 until around the stabilization point near day 34 the air bubbles gradually grew in size. In the next 30-day period the air bubbles slowly diminished in size until they had vanished, with the exception of some larger air pockets at the edge of the jar at day 64. At day 69 a sudden downward displacement was recorded giving a reading of −3.64 mm. This displacement gradually stabilized again around day 100, at a value of approximately −4.5 mm. Between day 295 and day 366 another downward displacement of −0.93 mm was recorded resulting at day 366 in a reading of −5.42 mm.

When comparing the temperature curve with the displacement curves, a relationship can be seen between the temperature readings and the displacement readings of the different systems. However, the measured changes in temperature do not significantly affect the trend of either displacement curve and are therefore not discussed further.

Table 2. Permeability constants (P) for polyethylmetacrylate (PEMA) in (cm³/m²·day·atm) from Ya-
suda and Stannett (1975).

Permeant	Temperature (°C)	P
H_2O	25	21,013
O_2	25	7.55
N_2	25	1.45

DISCUSSION

Absorption and permeability properties of Plexiglas®.—The outcome of the
absorption experiment shows that, with formaldehyde and glycerol solutions, wa-
ter is readily absorbed by Plexiglas®. Because of the presence of polar groups in
its chemical structure Plexiglas® has a relatively high affinity for water compared
to a plastic such as polyethylene, which is a hydrocarbon with no hydrophilic
substituents. This means that Plexiglas® has a higher permeability for water. High
water permeabilities are generally encountered with polar polymers or where the
segmental mobility is high, as for silicon rubber (Barrie 1968). The weight loss
of the sample soaked in 100% glycerol was probably caused by the desorption
of the water already present in the sample. Pure glycerol is apparently more
hydrophilic than Plexiglas®.

In conclusion, the loss of preservative fluid in Plexiglas® jars in our collection
is mainly caused by water in the preservative solution permeating through the
Plexiglas® to the outside. For this reason, in the pressure experiment and warping
experiment, only water was used to determine whether or not water loss by ab-
sorption and permeation in a sealed Plexiglas® jar could be responsible for the
pressure drop inside the jar and/or warping of its sides.

Negative pressure.—The pressure experiment shows that the loss of water by
diffusion causes an increased negative pressure inside the jar with respect to the
outside pressure. In our experimental model, after 1 mo a weight force of ap-
proximately 10 kg on an area of 10 by 10 cm was present. After approximately
3 mo this negative pressure had almost doubled. Although inside warping of the
top and bottom plates of the jar will have a leveling effect on the pressure increase
in time, the relatively high negative pressure and the pressure drop must be ex-
plained as the results of a dominant and fast absorption of water in the Plexiglas®
in the beginning and a much slower water loss by diffusion later.

The relatively high weight loss in the first period (days 1–110) compared to
the second period (days 111–204) does not completely support this idea, because
water absorption in the beginning would not be expected to result in weight loss
of the Plexiglas® container, whereas diffusion of the water to the outside envi-
ronment later would. Therefore, we expected a higher weight loss in the second
period than in the first. Experimental error in our test appears too large to allow
for a decisive conclusion. However, it is evident that the increase in negative
pressure is hardly compensated for by air influx, which can be explained by
looking at the permeability constants for water and air of polyethylmetacrylate
(PEMA), an acrylic similar to Plexiglas® (polymethylmetacrylate, PMMA) (Table
2). The value for water is approximately 15,000 times higher than that for nitrogen
and 3,000 times higher than that for oxygen, which means that air permeation

through these acrylics progresses at a much slower rate than does water permeation.

Because of the negative pressure inside the jar, the glued connections of the jar will be under considerable stress. Van Dam (2000) describes this phenomenon and suggested that the negative pressure can be avoided by placing a valve in the filling hole of the container, which vents the jar automatically in case of a pressure drop.

Warping of Plexiglas™.—In the warping experiment the Plexiglas™ lid of the open (vented) glass container warped to the inside in a relative short period (1 mo) and stayed nearly stable during the remainder of the experiment. We assume that this type of warping is caused by the swelling of the Plexiglas™ resulting from water absorption. Initially, the swelling starts at the inside surface of the Plexiglas™, progressing slowly through to the outside surface. Because of the difference in expansion of the inner swollen layer and outer unswollen layer, the direction of warping is to the inside. When full absorption is reached, there is no longer a difference in expansion between inside and outside layers, and thus we would expect the Plexiglas™ lid to return to its flat state. However, in our experiment the extent of warping did not change because the swollen Plexiglas™ lid was glued to the rim of the glass container, which prevented it from assuming its original form. If this assumption is correct, it would mean that with open, full Plexiglas™ jars, the warping would be temporary, until the maximum water absorption has been reached, because all sides undergo uniform expansion. On the other hand, it seems more likely that, after full absorption, the top layer of the outside surface would swell less as a result of the loss of water to the dryer outside atmosphere. In this case, warping by absorption in full Plexiglas™ jars will remain consistent.

The warping experiment with the closed container showed, contrary to what was expected, a gradual upward displacement of the lid from day 1 until day 35. This could have been caused by the formation of air bubbles underneath the lid, which were primarily dissolved in the water. Another explanation could be the expansion by swelling of the glued Plexiglas™ lid. Because of the incompressible water underneath, this would leave it no other direction to warp than to the outside, resulting in a larger jar volume. Consequently, the air bubbles underneath the lid would increase in size.

The sudden inward collapse of the lid between day 65 and day 70 resulted in a displacement reading close to the reading of the open container. We assume that fluid loss by diffusion or air loss by an undetected small leak in the closed container caused a decrease of the water/air volume to such an extent that the swollen lid could warp to a position identical to that of the open container. Between day 150 and day 300 the displacement readings of the closed and open containers are almost identical and seem to be stable. At this time, there was no evidence that the process of warping in the closed container could be explained by negative pressure inside the jar. However, between day 295 and day 366, the closed container showed another increase of approximately 1 mm downward displacement of the lid. At this time, the closed container showed that, evidently, a pressure drop resulting from the permeation of water through the Plexiglas™ lid resulted in a further increase of the displacement of the lid. Also, the warping in the closed container was not a gradual process, which would give the deformable Plexiglas™

the opportunity to adapt, but a process of relatively long periods of stability disturbed by sudden collapses. Such collapses easily can lead to the sudden bursting of the jar. In fact, in our opinion, fluid-filled sealed Plexiglas™ jars can be seen as ticking time bombs, which can implode at any moment, unexpectedly.

Solutions.—The use of thicker sheet material in constructing the jars does provide for stronger bonds, although it does not prevent negative pressure from occurring. On the contrary, thick-walled Plexiglas™ jars will initially have a higher water loss, caused by the relatively fast process of absorption, and they will have less ability to warp inside to compensate for the increase in negative pressure than will thin-walled jars. On the other hand, the rate of water loss by diffusion of thin-walled jars will be higher. Therefore, neither option will prevent the inevitable cracking of the jars over time.

Because the increase in negative pressure is the most important cause for the extreme warping and cracking of Plexiglas™ jars, the best method would be to prevent a pressure drop inside the jars. The use of a valve, which vents the jar automatically in case of a pressure drop, seems to be good practical solution. A disadvantage of the use of a valve is that the jar loses a part of its function as an oxygen barrier, which may speed up oxidation processes inside the jar (van Dam 2000).

A pressure drop inside the jar can also be avoided when there is no fluid loss by absorption and/or diffusion. When high concentrations of glycerin are used as a preservative fluid, it is possible to use a concentration that will compete with Plexiglas™ to absorb water. Table 1 shows that zero weight change for a Plexiglas™ sample can be found at a glycerol concentration between 67% and 100%. Theoretically, at such an equilibrium there would be no fluid loss, no pressure drop inside the jar, and consequently no warping. For formalin, the same result could be achieved by adding a hygroscopic salt to the solution. A disadvantage of these hydrophilic fluids is that they may also affect the preservation quality of the specimens.

Another method to prevent water loss would be to coat the Plexiglas™ with a substance that makes the jar impermeable to water. The possible use of coatings and hydrophilic preservative fluids should be further investigated.

The best solution is not to use Plexiglas™ jars at all. Despite their fragility, cast rectangular glass jars still seem to be the best alternative because of their impermeability for most chemical substances, including water and oxygen (van Dam 2000). However, at the present time the demand for these jars is very low and in the near future there is a good possibility that they will no longer be commercially available.

CONCLUSIONS

The causes for warping and cracking of sealed Plexiglas™ jars can be attributed to the water absorption and water permeability of Plexiglas™. A relatively fast initial water loss by absorption results in unequal hydration of the thickness of the Plexiglas™, followed by a slower rate of diffusion of water to the outside environment. This results in uneven swelling of the Plexiglas™ sides of the jar and an increase in negative pressure inside the jar, which causes the jar to warp to the inside and finally to crack at the glued joints. Solutions to these problems

may be found in the use of a valve, to avoid pressure drop inside the jar, the use of hydrophilic preservative fluids, or perhaps coatings to prevent water loss.

LITERATURE CITED

Barrie, J.A. 1968. Water in polymers. Pp. 259–313 in *Diffusion in Polymers* (J. Crank and G.S. Park, eds.). Academic Press, New York, New York. 452 pp.

van Dam, A.J. 2000. The interactions of preservative fluid, specimen container, and sealant in a fluid collection. Collection Forum 14(1–2):78–92.

Yasuda, H. and V. Stannett. 1975. Permeability coefficients. Pp. III229–III240 in *Polymer Handbook*, 2nd ed. (J. Brandrup and E.H. Immergut, eds.). John Wiley & Sons, New York, New York. 1,363 pp.

USE OF A LOW-OXYGEN ATMOSPHERE FOR THE CONTROL OF INSECT PESTS

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Abstract.—Eight species of stored-product insect pests at various stages of their development were exposed to a low-oxygen atmosphere of 1.5% for a period of 10 days. Apart from a 40% survival rate for the larval stage of *Anthrenus verbasci* (Coleoptera; Dermestidae), the modified atmosphere was observed to have a lethal effect on all insect stages tested. When the exposure period was extended to periods of 20, 30, 40, 50, and 60 days, 100% mortality was recorded for all insects tested. Evidence from this investigation supports the view that atmospheres reduced in oxygen may represent a viable alternative to chemical control methods. The feasibility of using this technique for the routine control and eradication of insect pests in museums is discussed.

Methods used for the eradication of insect pests in museum collections have traditionally involved the use of chemicals. Such chemicals may react adversely with museum materials and specimens, can be toxic to users, may be harmful to the environment, and their use is, in many cases, highly regulated (Dawson and Strang 1992, Jedrzejewska 1967, Linnie 1987, 1994). This has focussed attention on alternative methods of control. The use of carbon dioxide, low oxygen atmospheres, and inert gases (nitrogen, helium, and argon) have been considered alternatives to chemical control (Daniel et al. 1993). The advantage of the use of some of these atmospheric gases under controlled conditions is that the toxicity risk to users is reduced and the reactive effects on specimens and materials are considered less damaging than conventional chemical or fumigant treatments.

A modified atmosphere may be used to destroy or prevent infestations, as a control method in situations where infestations are suspected, and as a general "quarantine" treatment for incoming material. Modified atmospheres are created by replacing the existing ambient atmosphere with one lethal to insects. An environment low in oxygen may be achieved by adding carbon dioxide, nitrogen, or air depleted in oxygen under sealed conditions. This technique is a modification of the practice of hermetic storage where food commodities such as grain and beans were sealed in underground pits. The respiration of the harvested product combined with the metabolism of the invading insect pests reduced the available oxygen to a lethal level. The resulting atmosphere also inhibited fungal growth and maintained the quality of the food product over an extended period.

Although research into modified atmospheres began in the mid-19th century, interest in applying the technique in a routine manner did not occur until approximately 40 yr ago and serious interest about 20 yr ago, probably resulting from the success of and growing concern of health risks associated with conventional fumigants and grain protectants. Most recent research relates to the use of gases in silos, granaries, and other forms of bulk grain storage as a direct alternative to traditional chemical fumigation methods.

Studies have shown that the survival of stored-product insect pests is restricted in atmospheres containing low-oxygen concentrations (Navarro 1978, Rust et al. 1996, Soderstrom and Brandl 1982, Storey 1978). It has also been demonstrated

that to produce a lethal environment for even the most susceptible stored-product insect, the oxygen concentration should be maintained below 4% (Bailey 1965, Bailey and Banks 1974). Burke (1996) recommends concentrations of 0.5% or lower for museum insect pests and states that concentrations greater than 1% do not appear to be effective, even for longer exposures. Rust et al. (1996) found that the exposure time in a low-oxygen atmosphere is likely to be dramatically decreased by increasing the temperature above 25.5°C and lowering the relative humidity below 55%.

The application of modified atmospheres for the control of museum insect pests was first investigated approximately 10 yr ago. Since then, considerable work has been done on the efficacy of using the technique for the control of pest insects in museum collections (Elert and Maekawa 1997, Rust et al. 1996, Valentin 1993). Theoretically, the concept of exposing museum specimens to modified atmospheres should not present undue difficulties considering the technology available and standards achieved in ensuring safe chemical treatment by traditional fumigation methods. The advantage of low health risks combined with the residue-free benefits of the technique appear to offer a suitable alternative to chemical fumigation treatments (Daniel 1995).

In this study several species of recognised pests of stored food produce and some recognised pests of museum collections were exposed to a low-oxygen atmosphere over varying time periods. Where possible, egg, larval, pupal, and adult stages were used to determine potential susceptibility differences. An evaluation of the effectiveness of the technique is made as a pest-control tool in the museum environment.

MATERIALS AND METHODS

The selection of particular insect pest species was made on the basis of their recognised status as pests of stored dried-food produce and museums, their fecundity rates, and their availability. All of the species chosen are cosmopolitan and are considered among the most serious of the stored-product pests. In addition, seven of the species tested are included in a reference listing of museum pests (Beauchamp et al. 1981) and are known to feed directly on produce containing a high protein content. *Liposcelis bostrychophilus* (Psocoptera; Liposcelidae) does not appear on the list, although a related species, *Liposcelis simulans* is included. With the exception of two species, *Anthrenus verbasci* and *Dermestes maculatus* (Coleoptera; Dermestidae) that were selected from in-house laboratory cultures, all insects were purchased from the Central Science Laboratory, Slough, UK. Where possible egg, larval, pupal, and adult stages were used. A list of the species, experimental temperature conditions, numbers of individuals, and life-cycle stages used is given in Table 1.

The eight insect species selected for study were: *Dermestes maculatus* De Geer, Hide beetle (Coleoptera; Dermestidae); *Anthrenus verbasci* (L.), Varied carpet beetle (Coleoptera; Dermestidae); *Tribolium confusum* Jacquelin du Val, Confused flour beetle (Coleoptera; Tenebrionidae); *Tribolium castaneum* (Herbst.), Rust-red flour beetle (Coleoptera; Tenebrionidae); *Oryzaephilus surinamensis* (L.), Saw-toothed grain beetle (Coleoptera; Silvanidae); *Liposcelis bostrychophilus* Badonnel, Book-louse (Psocoptera; Liposcelidae); *Stegobium paniceum* (L.), Biscuit

Table 1. Percentage of mortality of various insect species following their exposure to a low-oxygen atmosphere for 10 days.

Species[a]	Stage	Number used	% Mortality at 20°C	% Mortality at 25°C
D. maculatus	adult	40	100	100
D. maculatus	pupa	40	25*	N/P
D. maculatus	larva	40	100	100
D. maculatus	egg	40	100	N/P
A. verbasci	adult	40	N/P	100
A. verbasci	larva	40	N/P	60
A. verbasci	pupa	30	N/P	100
A. verbasci	egg	40	N/P	100
O. surinamensis	adult	40	100	100
O. surinamensis	pupa	20	N/P	100
O. surinamensis	larva	40	N/P	100
O. surinamensis	egg	20	100	100
T. confusum	adult	40	100	100
T. confusum	pupa	40	100	100
T. confusum	larva	20	N/P	100
T. confusum	egg	20	N/P	100
T. castaneum	adult	40	100	100
T. castaneum	pupa	40	100	100
T. castaneum	larva	20	N/P	100
T. castaneum	egg	20	N/P	100
L. bostrychophilus	adult	40	100	100
L. bostrychophilus	egg	40	N/P	100
P. interpunctella	adult	20	100	100
P. interpunctella	larva	20	N/P	100
S. paniceum	adult	40	N/P	100
S. paniceum	pupa	30	N/P	100
S. paniceum	larva	40	N/P	100
S. paniceum	egg	20	N/P	100

* Failed to survive; N/P, not performed.
[a] D, Dermestes; A, Anthrenus; O, Oryzaephilus; T, Tribolium; L, Liposcelis; P, Plodia; S, Stegobium.

beetle or Drug-store beetle (Coleoptera; Anobiidae); and *Plodia interpunctella* (Hubner), Indian meal moth (Lepidoptera; Pyralidae).

Test Conditions

All the egg, larval, pupal, and adult stages were collected, sorted, and counted immediately prior to their exposure to the low-oxygen atmosphere. Eggs were examined under light microscopy to confirm that they were in good condition and probably viable. Larvae were chosen from laboratory cultures and, in all cases, final instar stages were preferred. Pupae were gently prodded with a blunt probe to confirm evidence of viability by their exhibition of a reflexive response to the stimuli of light and touch. Adult stages that were observed to be mobile and responsive were also selected from stock cultures. The precise numbers of individuals and the stages used for each species were dependent on their availability at the time of selection. Insects were retained in plastic petri dishes or in screw-cap jars, where appropriate, with small amounts of culture media until the insects' exposure to test conditions.

For their exposure to the low-oxygen atmosphere, each batch of test insects

was placed in a separate metal container with a small amount of culture medium. The containers were "tin" cans, approximately 1 L in capacity and measured 17 cm in height by 13 cm in diameter. The evacuation of oxygen from the cans involved an initial crimping and sealing of a metal lid onto each container. Air inside the cans was then removed using a "Terlet" industrial degassing unit that provides a negative pressure of 0.95 bar. Immediately after this process, nitrogen was added at a pressure of 1 bar, resulting in an atmosphere that was reduced in oxygen. This procedure was performed on-site in a commercial milk-powder man-ufacturing plant. The company uses low oxygen tension to increase the shelf life of their product. The concentrations of oxygen were initially recorded as 1.1, 1.6, and 1.3%. A second set of concentrations (taken 30 min later) were recorded as 1.5, 2.0, and 1.6%, respectively. The mean oxygen concentration was 1.5%.

Groups of control insects were maintained under identical conditions but were held in cans containing an ambient atmosphere. All cans were transported to the laboratory and maintained under constant temperature conditions at either 20°C or 25°C depending on each species' requirements. After 10 days the cans were opened for inspection. Death was determined by the absence of spontaneous movement or evidence of an irreversible, uncontrolled, lethargic condition in lar-val or adult stages. However, these criteria cannot be used for egg and pupal stages, therefore the cessation of normal development followed by obvious phys-ical deterioration was considered an indication of death for egg and pupal stages. All insect stages, whether considered viable or moribund, were held at either 20°C or 25°C (depending on the species) for monitoring. This was performed at 7-day intervals over a period of 3 mo.

RESULTS

Upon opening the containers with the low-oxygen atmosphere, it was evident that most insect stages had failed to survive. In contrast, control insects that had been held under ambient atmospheric conditions exhibited normal behavioural activity. Table 1 shows the percentage of mortality of the various insect stages and species following their exposure to the low-oxygen tension for a period of 10 days.

The only stages that exhibited any indications of viability were pupal stages of *D. maculatus* and larval stages of *A. verbasci*. Upon initial inspection, the pupae of *D. maculatus* appeared dead, although there was a reflexive response to gentle touching with a blunt probe. Subsequent monitoring indicated signs of further development, including the progression of elytral formation and head capsule growth. However, full development was impaired and none of the pupae pro-gressed to the adult stage. They were finally diagnosed as dead after the 3-mo monitoring period had elapsed and when emergence had been completed in the control samples.

Larval stages of *A. verbasci* exposed to the low oxygen atmosphere, although not mobile on initial inspection, responded to gentle touching with a blunt probe. Forty percent of larvae continued to improve and eventually became capable of complete and unimpaired movement. Development continued into the pupal stage and subsequent emerging adults appeared normal. All other insect stages failed to show any sign of viability during the 6-wk monitoring period. At the end of

Table 2. Percentage of mortality of mature larvae of *Anthrenus verbasci* (L.) following their exposure to a low-oxygen atmosphere over a variety of time periods at 25°C.

Exposure period (days)	% Mortality
10	60
20	100
30	100
40	100
50	100
60	100

this period a final assessment of their condition was made using the methods described above and by comparison with the control samples.

Of all the species and life-cycle stages tested, larvae of *A. verbasci* were the only insects to survive exposure to the test conditions. This ability was further investigated by repeating the experimental procedure; although in this case, the period during which the test insects remained in the low-oxygen atmosphere was extended to periods 20, 30, 40, 50, or 60 days at 25°C. Test conditions and oxygen tensions were maintained as described above. Forty mature larvae were used for each time period. Each test was duplicated and control tests using larvae held under the same conditions but with cans filled with ambient atmospheric air were also performed.

After each tested time period had elapsed, the test cans and the control cans were opened and inspected. Mortality in the control samples, even after 60 days in the sealed cans was negligible with larvae exhibiting normal behaviour such as movement and response to light and touch. However, all larvae exposed to the low-oxygen atmosphere over these time periods appeared moribund on removal from the deoxygenated containers. Subsequent monitoring of the test larvae over a 6-mo period following removal from the test conditions failed to record any survivors. Larvae were finally diagnosed as dead after the completion of the monitoring period or when emergence was completed in the control samples (Table 2).

DISCUSSION

The use of modified atmospheres to control pests has largely developed in response to demands by the food industry to produce methods of control that are nontoxic and residue free. Similarly, the disadvantages associated with the use of chemicals in the museum environment have promoted interest in alternative non-chemical control methods. Although the use of sealed environments low in oxygen to control museum insect pests has attracted considerable interest, further research is required to investigate fully, those factors that may assist or promote the survival of insects in anoxic conditions.

These results show that in general, exposure to an atmosphere of approximately 1.5% oxygen and 98.5% nitrogen for a period of 10 days was successful in limiting survival of the test insect species among the various stages used. Adults of *T. confusum, T. castaneum, D. maculatus, A. verbasci, O. surinamensis, L. bostrychopilus, P. interpunctella,* and *S. paniceum* all failed to survive the reduced oxygen atmosphere. Of the pre-adult stages tested, larvae of *A. verbasci* were the

only stage to survive the treatment with 40% eventually making a full recovery. Further tests on larvae of this species over extended time periods showed that an exposure period of 20 or more days achieved 100% mortality.

All insects require oxygen to survive and continue normal development and activity. The uptake and transport of oxygen from the surrounding atmosphere into the insect respiratory system is related to the ability of the insect to tolerate certain conditions. Given that certain stored-product insects can survive at very low humidities, the means of conserving water is an important and fundamental feature of their physiology and structure. In effect, this means that insects that have adapted to survive under dry conditions, such as those found in the bulk storage of grain and other food products, require efficient respiratory systems that allow rapid exchanges of oxygen and carbon dioxide while at the same time restricting water loss. This is an important consideration because anoxia in insects works by dehydration rather than by suffocation. This has also been suggested by other workers, where water loss was found to be a major contributory factor in the mortality of *T. castaneum* held under high nitrogen concentrations (Jay 1971, Jay and Cuff 1981). At very low oxygen concentrations the spiracles of the insect open to such an extent that desiccation occurs. Significant weight loss caused by desiccation in certain insect larvae exposed to a range of modified atmospheres has been reported (Valentin 1993). This condition is further accelerated by dry, warm conditions. In contrast, if ambient humidity is high and combined with low temperature, the insect may receive sufficient moisture to avoid death and render anoxia unsuccessful as a method of control.

In the culture conditions described here, *A. verbasci* did not require direct contact with water, deriving sufficient moisture requirements from the surrounding media. This ability to survive very dry conditions may have contributed to the survival rate. Another factor which may have contributed to the survival rates in *A. verbasci* may be the ability to enter a diapause state when conditions become unfavourable for further development. This facility has been defined by Hanski (1988) as an arrested development phase that minimises the utilisation of body reserves, thereby reducing the risk of an individual dying as a result of unfavourable conditions. This ability provides a natural defence mechanism during conditions of particular hardship and can persist for considerable time periods. In natural populations diapause typically occurs in response to unsuitable ambient temperature or food scarcity.

The results of this study indicate that the use of an atmosphere reduced in oxygen has a lethal effect on a range of insect species. The main difficulty facing conservators is the application of the correct combination of oxygen concentration, exposure, temperature, and humidity required to achieve a lethal effect on the particular pest species under treatment. Beauchamp et al. (1981) in a reference listing of museum pests, loosely describe 123 pests of museums, of which 98 are insects. The variability in response of different insect species to different concentrations of oxygen indicates that no general conclusions can be drawn; i.e., the response of one species cannot be used as an indicator of the response of other species. Also, other potential museum pests, such as mites, typically occupy relatively dry habitats, possess a highly specialised cuticle to prevent water loss, and the spiracles are able to regulate gas exchange through a complicated mechanism

resulting in low water loss rates and good osmoregulatory ability (Fleurat-Lessard 1990).

Until recently, modified atmospheric conditions have been applied mostly to controlling insects in large bulk materials containing dry grain or cereals. These methods have also proved useful in destroying infestations where chemical insecticide control was considered unsuitable or impractical. The apparent success of the use of modified atmospheres for the control of stored-product insects has led to interest among museum workers as to whether the technique could be successfully applied to pests of museum collections. Recent advances in the development of oxygen scavengers, oxygen barrier films, and fumigation "bubbles" have facilitated this trend and several museums are now using the technique routinely (Elert and Maekawa 1997, Gilberg and Roach 1992, Maekawa and Elert 1996, Valentin 1993).

Oxygen-absorbent chemicals, when used in sealed conditions, have been shown to be effective against certain pests of stored products (Burke 1996, Ohguchi et al. 1983). One such commercially available product is the oxygen scavenger "Ageless" manufactured by the Mitsubishi Gas Chemical Company of Japan. Ageless is prepared from powdered iron oxide and is manufactured in packet form, designated according to type and size. Gilberg and Roach (1992) describe the successful use of Ageless at the Australian Museum. Objects are packaged within a flexible, low-oxygen permeability barrier film along with the requisite amount of Ageless, heat sealed, and placed within a temperature controlled cabinet for 3 wk at 30°C. Subsequent examination of infested objects failed to indicate any evidence of continued insect activity.

The use of oxygen scavengers is limited to objects of small to moderate size. For the treatment of large items, alternative methods of producing a modified atmosphere lethal to insects is required. These methods rely on creating an airtight enclosure that is flushed with a humidity-controlled nitrogen source to achieve the desired low-oxygen level. The technique may be used as an alternative to subzero temperature control, where the size of material requiring treatment limits freezing by conventional methods or where concerns of potential deleterious effects to specimens and materials may limit its use. Specific applications that may be appropriate include treatment of infested bulk items and as a quarantine treatment for incoming material. The technique may be usefully employed in conditions that are gas proof, such as fumigation chambers or in areas that can be sealed against gas loss. Gas-proof fumigation sheets may also be suitable and the recent development of a fumigation "bubble" by Rentokil Ltd. that is portable and has a built-in inlet and venting system is a promising development. The "bubble" was originally manufactured in 1988 for use with toxic fumigants such as methyl bromide but was later redesigned for use with nitrogen and consists of a heat-sealable, aluminised barrier film. Maekawa and Elert (1996) describe the use of an anoxic treatment system for the disinfestation of museum objects. The system consists of a high-volume nitrogen source, a gas humidification module, the anoxic enclosure, environmental sensors, and a vacuum pump. The system had a leak rate of <0.005% (50 ppm), and this was found sufficient to maintain the required anoxic environment with no nitrogen flow for several weeks once an initial oxygen level of 0.1% had been achieved.

The use of carbon dioxide should also be investigated, because it has been

shown to be effective at levels as low as 30% (Story 1985). This makes it less difficult to maintain at a level toxic to insects than other gases such as oxygen, that require a level <2%. However, the use of carbon dioxide requires special handling, because as little as a 2% rise in air concentration can cause increased respiration in humans. A self-contained breathing apparatus is therefore essential before entering areas undergoing carbon dioxide fumigation. For this reason, reduced oxygen atmospheres may be more appropriate for museums when treatment of bulk items is necessary.

Although further research is required, low-oxygen atmospheres offer the potential of effective, residue-free pest control, combined with a reduced environmental hazard, providing a viable alternative to chemical fumigation. The advent of plastic barrier films of low-oxygen permeability combined with commercially available oxygen absorbent agents, facilitate the ease of use for most museums. Similarly, the use of air-tight enclosures under anoxic conditions are now well documented and represent a welcome development for the in-house treatment of bulk items.

LITERATURE CITED

Bailey, S.W. 1965. Air tight storage of grain: Its effect on insect pests, IV. *Rhyzopertha dominica* (F.) and some other Coleoptera that infest grain. Journal of Stored Products Research 1:25–33.

Bailey, S.W. and H.J. Banks. 1974. The use of controlled atmospheres for the storage of grain. Pp. 375–382 in *Proceedings for the 1st International Working Conference on Stored Product Entomology.* Savannah, Georgia.

Beauchamp, R., J. Kingslover, and T. Parker. 1981. Appendix C: Reference listing of museum pests. Pp. C1–C5 in *Pest Control in Museums: A Status Report (1980).* (S.R. Edwards, B.M. Bell, and M.E. King, eds.). Association of Systematics Collections, Lawrence, Kansas. 102 pp.

Burke, J. 1996. Anoxic microenvironments: A simple guide. Society for the Preservation of Natural History Collections, SPNHC Leaflets 1(1):1–4.

Daniel, V. 1995. Storage in low-oxygen environments. Pp. 147–155 in *Storage of Natural History Collections: A Preventative Conservation Approach* (Rose, C.L., C.A. Hawks, and H.H. Genoways, eds.). Society for the Preservation of Natural History Collections, New York, Pennsylvania. 448 pp.

Daniel, V., G. Hanlon, and S. Maekawa. 1993. Eradication of insect pests in museums using nitrogen. WAAC Newsletter 15(3):15–19.

Dawson, J.E. and T.J.K. Strang. 1992. Solving museum insect problems: Chemical control. Canadian Conservation Institute Technical Bulletin 15:1–26.

Elert, K. and S. Maekawa. 1997. Rentokil bubble in nitrogen anoxia treatment of museum pests. Studies in Conservation 42:247–252.

Fleurat-Lessard, F. 1990. Effect of modified atmospheres on insects and mites infesting stored products. Pp. 21–38 in *Food Preservation by Modified Atmospheres* (M. Calderon and R. Barkai-Golan, eds.). CRC Press, Florida.

Gilberg, M. and A. Roach. 1992. Inert atmosphere disinfestation of museum objects using Ageless oxygen absorber. Pp. 397–406 in *Biodeterioration of Cultural Property 2; Proceedings of the 2nd International Conference on Biodeterioration of Cultural Property.* International Congress of Biodeterioration of Cultural Properties, Yokohama.

Hanski, I. 1988. Four kinds of extra long diapause in insects: A review of theory and observations. Annales Zoologici Fennici 25:37–53.

Jay, E.G. 1971. Susceptibility of two species of *Tribolium* (Coleoptera: Tenebrionidae) to alterations of atmospheric gas concentrations. Journal of Stored Products Research 7(3):181–186.

Jay, E.G. and W. Cuff. 1981. Weight loss and mortality of three life stages of *Tribolium castaneum* (Herbst) when exposed to four modified atmospheres. Journal of Stored Products Research 17: 117–124.

Jedrzejewska, H. 1967. The damaging influence of disinfecting agents on sensitive ancient materials. Pp. 95–101 in *Contributions to the London Conference on Museum Climatology* (G. Thompson, ed.). International Institute for Conservation of Historic and Artistic Works, London, UK. 296 pp.

Linnie, M.J. 1987. Pest control: A survey of natural history museums in Great Britain and Ireland. The International Journal of Museum Management and Curatorship 6:277–290.

Linnie, M.J. 1994. Pest control in natural history museums: A world survey. Journal of Biological Curation 1(5) 1993(1994):43–58.

Maekawa, S. and K. Elert. 1996. Large-scale disinfestation of museum objects using nitrogen anoxia. Pp. 48–53 in Preprints, 1, ICOM Committee for Conservation, 11th Triennial Meeting, Edinburgh, Scotland (J. Bridgland, ed.). James and James, London, UK.

Navarro, S. 1978. The effects of low oxygen tensions on three stored-product pests. Phytoparasitica 6(2):51–58.

Ohguchi, Y., H. Suzuki, S. Tatsuki, and J. Fukami. 1983. Lethal effect of an oxygen absorber on several stored grain and clothes pest insects. Japanese Journal of Applied Entomology and Zoology 27:270–275.

Rust, M.K., V. Daniel, J.R. Druzik, and F.D. Preusser. 1996. The feasibility of using modified atmospheres to control insect pests in museums. Restaurator 17:43–60.

Soderstrom, E.L. and D.G. Brandl. 1982. Antifeeding effect of modified atmospheres on larvae of the navel orangeworm and Indian meal moth (Lepidoptera: Pyralidae). Journal of Economic Entomology 75(4):704–705.

Storey, C.L. 1978. Effect of low oxygen atmospheres on mortality of red and confused flour beetles. Journal of Economic Entomology 70(2):253–255.

Story, K.O. 1985. *Approaches to Pest Management in Museums.* Conservation Analytical Laboratory, Smithsonian Institution, Suitland, Maryland. 165 pp.

Valentin, N. 1993. Comparative analysis of insect control by nitrogen, argon and carbon dioxide in museums, archive and herbarium collections. International Biodeterioration and Biodegradation 32:263–278.

SPIRIT COLLECTIONS: ACCELERATED AGING STUDIES CONCERNING THE STABILITY OF KERATIN IN ETHANOL AND FORMALIN

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Abstract.—The keratins are a closely related family of chemically stable proteins composing mammalian hair, horn, hooves, and avian feathers. Sheep hair (wool) has been much studied chemically because of its economic value. To our knowledge, no studies have been conducted on the long-term stability of other keratins from a museum perspective. We present here differences in the stability of feathers and hair under simulated aging conditions. Feathers and hair were heated dry, in 70% ethanol, and in 70% ethanol plus 1% formalin at 180°C for periods of 1 and 2 days. Feather keratin was approximately 50% less stable in ethanol than was hair keratin, as evidenced by the amount of amino acids lost from the sample and appearing in the solution. Feathers and hair, heated for the same periods of time under dry conditions, exhibited the same pattern of stability. As a corollary, the amino acid patterns of fresh hair and feathers from our samples of different species were found to be distinct and indicative of their originating taxon.

The word "keratin" denotes a class of linear structural proteins that is characterized by disulfide bonds that link the sulfur atoms of two cysteine amino-acid residues. To a large degree, the stability of keratin is dependent on these bonds. Keratins are found primarily in the epidermal regions of vertebrate animals and function in a protecting capacity. This class of proteins encompasses the outer layer of skin, hair and fur, horn, nails (and hooves), feathers, and bird beaks (Yu et al. 1993).

Keratins are pervasive in natural science and anthropological collections and are stored either "wet" or dry. Mammalian pelts and bird skins are stored dry, as are many cultural objects made from keratin. Entire bodies of animals and animal parts are maintained for research and taxonomic purposes. These are stored both as dried specimens and preserved in fluids. Despite this widespread presence in natural history museums, a protocol for keratin storage has yet to be developed. The variety of "dry" conditions includes storage at a constant room temperature and moderate relative humidity, as well as refrigeration in cold storage vaults at various temperatures and relative humidities. Fluid storage involves putting keratinous material in mixtures of reactive chemicals such as ethanol and formalin and often involves "topping off" when the level of liquid in the specimen container decreases from evaporation. This addition of fluid has an unknown effect on the possible leaching of chemicals from the specimen (von Endt 1994).

For these reasons, and because the chemistry of keratin is relatively obscure, optimal storage conditions are unknown. The effects of such factors as temperature and water vapor must be researched and controlled to ensure the integrity of stored specimens and artifacts of scientific and historical significance. Before optimal storage conditions can be determined, the nature of keratin itself must be examined. It is important to understand the chemical reactions undergone by ker-

atin, as well as the mechanisms by which it deteriorates. Because its disulfide bonds characterize keratin, it should be possible to examine specific proteinaceous materials such as hair and feathers and apply the resulting data to the class of keratins as a whole. Because there are some structural similarities between keratin and collagen, another structural protein of great importance in natural science collections, some of the information obtained by studying keratin may be applicable to the properties of collagen as well.

The Nature of Keratin

Keratins appear in many forms in all vertebrate phyla (Menefee 1977). Keratins are separated into "soft" and "hard" categories. Soft keratins encompass the bulk of the relatively low-sulfur-containing outer layer of skin, whereas the hard keratins include the relatively high-sulfur-containing hair, hoof, nail, feather, and bird beak (Arai et al. 1993, Yu et al. 1993).

Keratins are significant for their insolubility (as are other structural proteins such as mature collagen). They have developed biologically as "a mechanically tough protective coat" (Menefee 1977). There are three primary methods by which fibrous keratin macromolecules are stabilized: (a) crystallization between polymer chains may occur biologically, (b) disulfide crosslinks between cysteine molecules may be formed to produce the amino acid cystine (and keratins characteristically contain extensive intermolecular cystine crosslinking) (Arai et al. 1993, Menefee 1977, Mercer 1961), and (c) covalent crosslinks between polypeptide chains may be introduced by chemical processes (such as tanning).

The primary structure of keratin consists of a molecular backbone formed by amino acids covalently linked by peptide bonds between the N-terminus, or amino end of one molecule, and the C-terminus, or carboxylic acid end of another molecule. The secondary structure of keratin results primarily from hydrogen bonding between amino-acid molecules and is an alpha helix. However, if the filament is stretched, it converts from an alpha helix to a beta-pleated sheet. In the case of keratin, a tertiary structure results from disulfide bonds linking cysteines located across alpha helices from one another. The most complex spatial arrangement of keratin, the quaternary structure, involves the interaction of multiple amino-acid chains. This quaternary conformation resembles a coiled-coil rope, often called a supercoil (James et al. 1995, Steinert et al. 1994, Tucker et al. 1989, Wilk et al. 1995).

Some of the chemistry of keratin reactivity is still somewhat unclear. However, not only do many old keratin artifacts exist in museums that indicate keratin stability, but under mild laboratory conditions the protein is quite insoluble because of its disulfide crosslinks, which implies that the potential for successful fluid storage also exists.

Previous Research

To our knowledge, no information is available concerning the long-term stability of keratin in natural history collections, especially of keratin stored in fluids. It appears to be unstable, however, because few, if any, existing mammalian skins in collections were collected prior to 1840 (C. Hawks pers. comm. 1994). To some degree, this observation about skins may also be the result of specimens deteriorating to the point where they are deemed no longer useful in the collection

(perhaps because of pest damage prior to the widespread use of pesticides) and are discarded.

The medical and law enforcement professions have studied hair, while the textile industry has advanced the study of wool. The Commonwealth Scientific and Industrial Research Organization (CSIRO) in Australia has been the primary source of wool research (Dowling and Sparrow 1991).

There have been four major directions in modern keratin research. Initial studies used light- and scanning-electron microscopy to describe the physical appearance of the hair and feathers themselves. These methods have been used by law enforcement agencies to catalog the microscopic and morphological features of hair from different species of animals (Hicks 1977) and to document the feather morphology of different species of birds (R. Laybourne pers. comm. 1994).

The molecular structure of keratin fibers has been examined using methods such as x-ray diffraction and infrared spectroscopy. Crewther et al. (1965) state that the "elucidation of the structure of alpha-keratin is a task of such enormous complexity that it has stimulated a great deal of research. . . ." The accepted structure for alpha keratin consists of multiple helices coiled around two central filaments, with the entire supercoil held together by disulfide bridges and hydrogen bonds (Parry et al. 1977). Although x-ray diffraction and infrared spectroscopy cannot provide structural models (Crewther et al. 1965), they can confirm or refute proposed models. The supercoil satisfies the data supplied by these analytical methods. For a detailed discussion of the structure of keratin, see the review articles by Crewther et al. (1965) and Bradbury (1973).

A third focus of keratin research pertains to the mechanical properties of wool fibers and feathers (Bonser and Purslow 1995, Wortman and Zahn 1994). The mechanical properties of wool have been studied by the textile, clothing, and hairdressing industries, which are concerned with the processes that occur as the fiber is stretched, set, and supercontracted. The physical alterations caused by the stress of stretching result in a conversion from the alpha-helix conformation to the beta-pleated sheet conformation, a change in the secondary structure of the protein. The supercontraction of a fiber is the shrinkage that takes place when it is stretched through setting, then steamed again and allowed to contract. This phenomenon differs from the contraction that occurs when a fiber is stretched and allowed to return to its original state without additional treatment. The effects on the measurement of these three mechanical properties when the fiber is soaked in water has been examined also (Bradbury 1973, Crewther et al. 1965).

The fourth series of studies involves protein sequencing and has been a primary focus of researchers at CSIRO. By 1991, 28 of the more than 100 proteins in wool fibers had been sequenced, encompassing about 5,000 amino-acid molecules. This represents an estimated 60–80% of the protein content of wool based on weight (Dowling and Sparrow 1991). With advances in genome mapping, however, "it is likely that any further wool protein sequences will be determined via DNA technology" (Dowling and Sparrow 1991) and will not involve sequencing the protein directly by the sequential removal of amino acid molecules.

Some studies of protein deterioration have been conducted that relate to keratin, but have not dealt with protein deterioration specifically, and therefore their relevance is unknown (Whitaker and Fujimaki 1980). Proteins can undergo hydrolysis (with water or water vapor), catalyzed by small amounts of either acid or

base. Amino acid amide groups and disulfide bonds are susceptible to attack under alkaline (higher pH) conditions. Alkaline conditions also can cause amino acid racemization, elimination reactions, the formation of new, destabilizing products such as lysinoalanine, and the formation of degradation products such as dehydroalanine. In addition, the disulfide bonds of cystine (an amino acid composed of two cysteine molecules bonded across their respective sulfur atoms) are susceptible to reduction, whereas both the S-methyl group of methionine and the thiol group of cysteine are susceptible to oxidation (Whitaker and Fujimaki 1980).

Keratin can be completely denatured and solubilized with urea and mercaptoethanol (Means and Feeney 1971), but this method may progress beyond dissolution, break peptide bonds, and destroy important chemical information. A more gentle method of solubilizing keratin while retaining more of its integrity involves breaking the disulfide bonds by either oxidation or reduction reactions. If left alone however, the disulfide bonds will regenerate themselves but in different ways. By converting the reactive sulfur atoms to thiols or sulfonyls, recreation of the disulfide bridges can be prevented. It is therefore common practice to oxidize or reduce the bond and then alkylate it. This solubilizes the protein, allowing its preparation for analysis, in addition to retaining as much chemical information as possible.

MATERIALS AND METHODS

Samples of feathers were collected primarily as molts from captive parrots and parakeets, supplied by a local pet owner. Samples of hair for the heating experiments were taken fresh from the tail of a horse quartered at a local stable, and from a dog, cat, guinea pig, and rabbit from a local veterinarian. Volunteers supplied human hair.

For the "wet" samples, approximately 1 mg of hair and feather was cut, weighed, and placed into 10 × 150 mm Pyrex® test tubes that contained 1 ml of solution. The "dry" samples were placed into empty tubes. Simulated storage solutions were formulated from 70% grain (ethyl) alcohol that was diluted from material purchased as a 95% solution from a local liquor store. Grain alcohol was chosen, because it contains no stabilizers, in contrast to laboratory alcohol. Solutions of ethanol and formalin were made by adding 70% ethanol to 1 ml of a 37% formaldehyde solution to a final volume of 100 ml of solution. Tubes containing the samples and solutions then were sealed in a flame and placed into a laboratory oven maintained at $180 \pm 1°C$. At specified time intervals, the tubes were removed from the oven, opened, and any remaining solid sample removed, dried, and weighed to determine weight loss during the experiment. The liquid was dried in the tube over silica gel under vacuum. The dried liquid and their respective hair or feather sample then were sealed separately in tubes containing 200 μl of 6 N hydrochloric acid, flushed three times with nitrogen to remove any oxygen and prevent sample oxidation, and then heated for 20 hr at 100°C to hydrolyze proteins and peptides to amino acids. The hydrolyzed samples then were dried over silica gel under vacuum and placed into a sample vial containing 0.25 M sodium citrate solution at pH 3.2, prior to introduction into the analyzer.

Amino-acid analysis was conducted on a specially constructed high-pressure liquid chromatograph, similar to the one described by Benson and Hare (1975) and by von Endt (1994) in its current modified form. Briefly stated, a 2-mm

internal diameter (i.d.) × 150-mm long stainless-steel column filled with 3 μm diameter cation exchange resin beads was connected to a series of buffer chambers via high-pressure tubing and a high-pressure piston pump. The chambers were maintained under 1.7×10^5 pascals (25 psi) helium pressure. The pump drew buffers from the chambers in a timed sequence as determined by a sample changer/controller. Four of the separating buffers consisted of 0.25 M sodium citrate of differing pH, each containing 1 g/L of ethylenediaminetetraacetic acid. The final buffer was a 0.2 M boric acid solution containing 1 g/L sodium chloride. Buffers entered the column in a sequence of ascending pH: 3.25, 4.5, 6.5, and 10. The column effluent then was mixed with a stream of *O*-phthalaldehyde (OPA) and 2-mercaptoethanol as the detecting reagent. *O*-Phthalaldehyde reacts with amino acids containing primary amines (most amino acids) to produce an OPA/mercaptoethanol/amino-acid product that fluoresces under UV light. The fluorescence is detected, acquired, and recorded by a detector and data system. Two common amino acids contain secondary amines in a ring structure (proline and hydroxyproline) but do not react under these conditions, and are not detected.

RESULTS AND DISCUSSION

Modern hair from four mammalian species (human, dog, cat, and guinea pig) and feathers from two individuals of the same species of bird were examined for their relative amino-acid content using an amino-acid analyzer. The chromatograms of seven selected amino acids from each analysis, and a standard for comparison, are presented below in Figure 1A–F. The sample of feather keratin (Fig. 1B) differs from the group of mammalian hair samples, because it contains relatively greater proportions of aspartic acid (amino-acid peak 1), serine (peak 3), glycine (5), and valine (7). There are also noticeable differences in the amino-acid content of the mammalian hairs themselves. Human hair (Fig. 1E) is most closely related in amino-acid content to that from the dog (Fig. 1F) (this observation is not meant to imply a close phylogenetic linkage), but differs from it in that the amounts of serine (3) and glutamic acid (4) are more nearly equal to each other in the dog hair than in the human hair. Also, the dog hair contains slightly more glycine (5), relative to alanine (6), than does human hair, whereas the human hair contains slightly more serine (2), relative to aspartic acid (1), than does the dog. The guinea pig (Fig. 1C) and cat (1D) differ from each other especially in their relative amounts of serine (peak 3), glutamic acid (peak 4), and glycine (peak 5). Glutamic acid is present in greater amount than is serine in the guinea pig chromatogram; this chromatogram is unique in that regard. Although cat hair exhibits the same general serine/glutamic acid relationship, as do the mammals other than guinea pig, the relatively large amount of glycine (5) in cat hair helps set it apart from the other mammals. In summary, the mammalian hairs and feathers analyzed for this study, and the taxa they represent, exhibit amino-acid patterns that seem to allow each taxon sampled to be distinguished from the others.

In an early study, Darkus and Gillespie (1971) used electrophoretic data to distinguish among four genera of sheep and goats, but were not able to distinguish among individual breeds of sheep using this technique. Hrdy and Baden (1973) also indicated that there were no differences in the electrophoretic patterns of human hair they examined from six areas of the world, but they reported that

differences did exist at the family level for nonhuman primates. Amino-acid analysis also was unable to distinguish among the human hairs (no amino-acid data was presented for the nonhuman primates). Gillespie and Frenkel (1974) and Gillespie and Marshall (1977) noted, however, that the concentration of various amino acids did differ in echidna claw and quill, ox hair, horn, and hoof, and rabbit hair and claw, indicating that the potential does exist for using amino-acid analysis to distinguish among taxa.

The chromatographic results of each sample also can be adjusted to account for differences in detectability ("color yield") of the individual amino acids illustrated in the chromatogram of the standard (Fig. 1A). When this correction is made, a numerical value can be assigned to each amino acid in each sample, and the data can be compared in a quantitative manner. The results from treating the analyses in this manner are summarized below in Table 1. Here, each amino acid was normalized to glutamic acid (GLU), so that the values presented in the table are ratios with respect to GLU.

Examination of the amino-acid content of the hair and feather samples summarized in Table 1 reveals differences in the relative proportions of amino acids, as seen visually in Figure 1B–F. In addition, the contrasting relative amounts of the amino acids imply the presence of different keratin proteins in the feathers and hair and suggest that the chemical reactivity and hence decomposition may also be dissimilar and require different conditions for optimum storage.

Aging experiments simulating fluid storage, and using a single temperature, indicate that dramatic differences can be found in the stability of keratin heated in different fluids, and that an additional series of aging experiments using different temperatures and storage conditions are warranted to further explore the stability of keratin.

Figures 2–4, below, illustrate the stability differences of keratin heated in simulated storage fluids. In each of the figures, the upper chromatogram is an analysis of a sample of modern keratin (hair or feather). The lower part of each figure is a chromatogram of a portion of the modern sample artificially aged at 180°C and 24 hr either in 70% ethanol, or in 70% ethanol + 1% formalin. Further, each illustrated analysis represents the same original weight of sample, so that the results may be compared directly to one another.

Figure 2 illustrates the difference in amino-acid content between untreated hair (the upper portion of the figure) and hair heated at 180°C in 70% ethanol for 1 day (the lower portion of Fig. 2). First, notice that the millivolt scale on the left side of the figure indicates that the "treated" hair has lost about half of its amino acids. (Peak height [millivolt] readings can be used for comparison in this case because the samples are being compared in a more descriptive, semi-quantitative manner, as well as having been prepared using equal protein concentrations. Normally, quantification involves a comparison of the integrated area of each peak.) Figure 2 also indicates that the amino acids labeled A and B have disappeared in the heated sample, a new amino acid labeled C has appeared, and the proportions of all the amino acids have changed. Finally, the amount of ammonia (labeled D) in the "treated" hair has increased, indicating increased deterioration. Other changes can be seen in this area of the chromatogram, but represent other, unknown deterioration products.

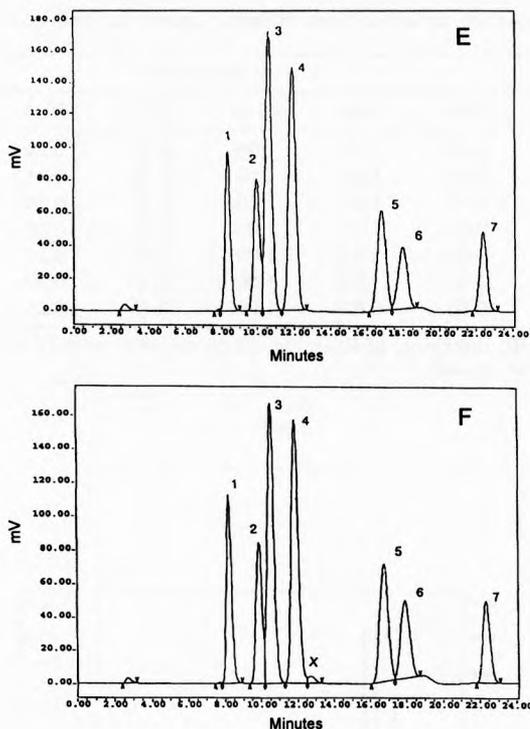The results of heating hair in the presence of a small amount (1%) of formalin
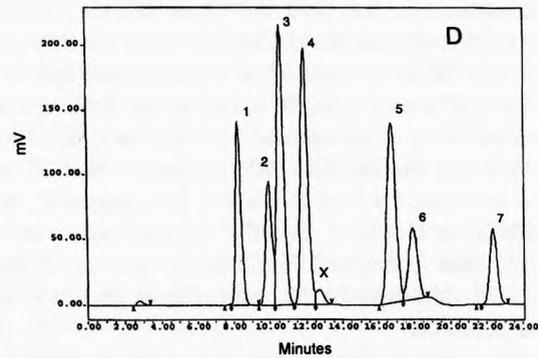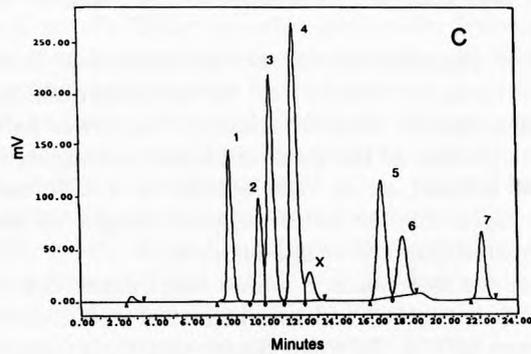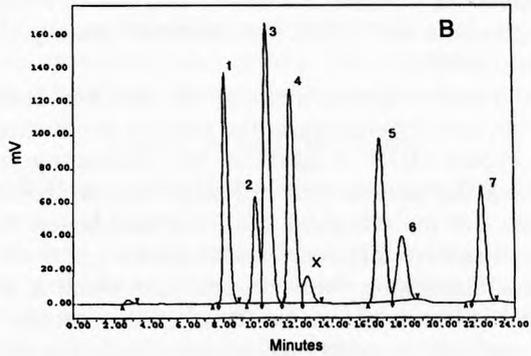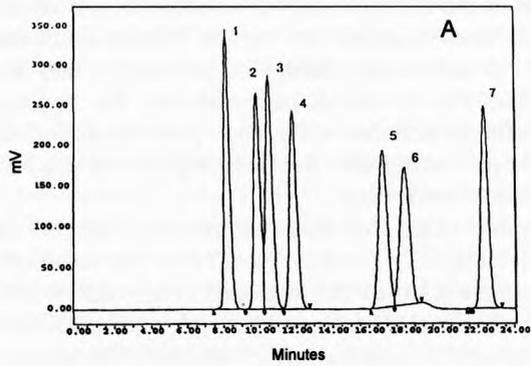
Figure 1. Continued.

in ethanol (to simulate the amount that might be found in fixed specimens) are illustrated in the lower portion of Figure 3 and can be compared to the analysis of the untreated sample above it. In Figure 3, virtually all amino acids have disappeared in the treated specimen, and the millivolt scales indicate that only about 10% of the hair remains. Figure 4 indicates that feather also is not stable when stored in ethanol. The millivolt scales show that feather heated in 70% ethanol alone has lost well over 50% of its amino acids (compared to hair in Fig. 1 that lost about 50%).

CONCLUSIONS AND FUTURE DIRECTIONS

On the basis of these data, a series of conclusions can be drawn. First, note that the keratins are unstable under artificial aging conditions using high temperatures. What is of interest here is the ability to compare the stability of keratin from different sources within a reasonable length of time. If one assumes that the reactions seen at these higher temperatures are the same general type that can be

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Figure 1.   All parts of this figure are chromatograms of seven amino acids generated by an amino acid analyzer. Peak 1 = aspartic acid, 2 = threonine, 3 = serine, 4 = glutamic acid, 5 = glycine, 6 = alanine, 7 = valine, and X = an unknown. Part A of the figure represents a standard mix of amino acids used for identification and quantification. Part B represents a parrot feather, C is guinea pig hair, D is cat hair, E is human hair, and part F, dog hair.

Table 1.   Selected amino acids in keratin from different sources normalized to glutamic acid.

Source	Amino acids							
	ASP	THR	SER	GLU	GLY	ALA	ILE	LEU
Feather A	1.44	0.62	1.81	1.00	0.37	0.29	0.12	0.46
Feather B	1.09	0.50	1.31	1.00	0.78	0.31	0.19	0.31
Carmen	0.68	0.55	1.07	1.00	0.36	0.26	0.09	0.26
Wendy	0.64	0.54	1.14	1.00	0.42	0.26	0.12	0.25
Horse	0.81	0.41	1.02	1.00	0.37	0.29	0.12	0.46
Cat	0.70	0.47	1.08	1.00	0.70	0.30	0.09	0.23
Guinea pig	0.54	0.36	0.80	1.00	0.43	0.25	0.08	0.21

ASP, aspartic acid; THR, threonine; SER, serine; GLU, glutamic acid; GLY, glycine; ALA, alanine; ILE, isoleucine; LEU, leucine.
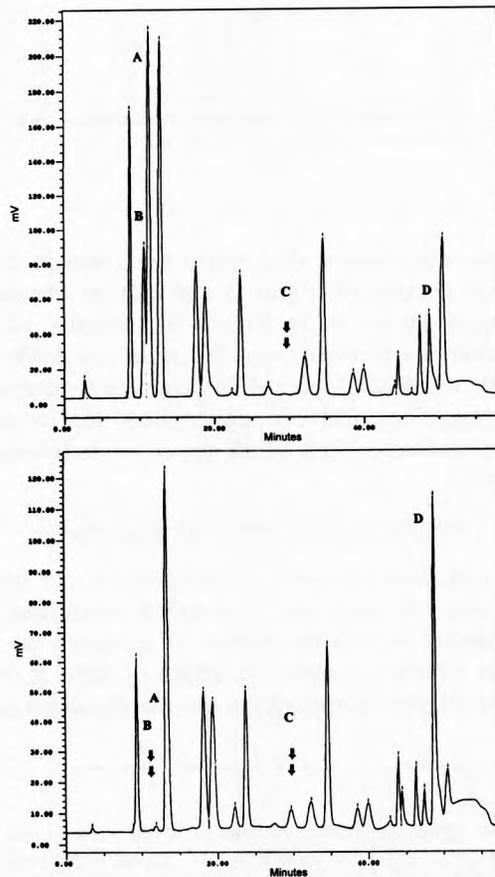
Figure 2.   An amino-acid analysis of modern hair (upper part of the figure). The lower portion is hair that has been heated in 70% ethanol at 180°C for 24 hr.
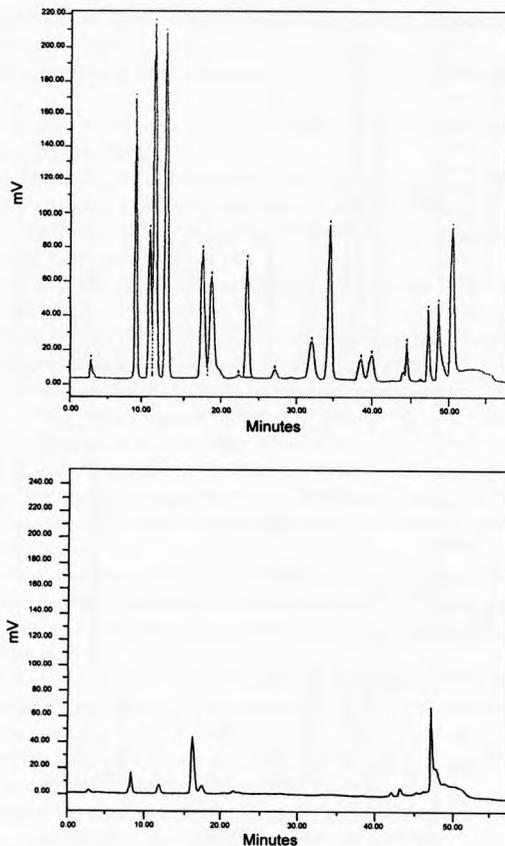
Figure 3.   An amino-acid analysis of modern hair (upper part of the figure) and hair heated at 180°C in 70% ethanol and 1% formalin for 24 hr.

observed at lower (room) temperature, then reasonable inferences can be drawn from these aging experiments and projected to museum conditions. One also can note that feather is less stable than hair.

When keratins are "stored dry" they last longest. They last less long in 70% ethanol, and least long in 70% ethanol + 1% formalin. These observations imply that even small amounts of formalin promote deterioration reactions in the keratins, seen especially dramatically under the experimental conditions described above. Further, under these experimental conditions, the difference between "longest" and "least long," as seen in the chromatographic data is several orders of magnitude. The chromatographic and tabular data also indicate that keratins are compositionally distinguishable among taxa.

By comparing the rate of change in amino acids at several temperatures under several "storage conditions," such as dry, 70% ethanol, and 70% ethanol plus 1% formalin, it should be possible to assign a numerical value to a rate of change that can be related to storage temperature as well as storage medium. This information then can be used to predict an expected "lifetime" of keratin under specific conditions of storage at a specific temperature. These data then can be com-
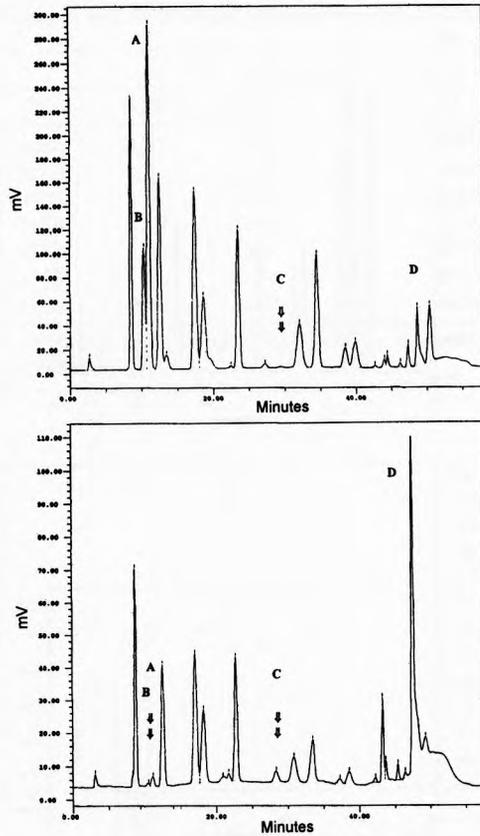
Figure 4. An amino acid analysis of modern feather (upper part of the figure) and feather heated in 70% ethanol at 180°C for 24 hr.

pared to that from actual museum specimens, and lead us not only to a better understanding of the stability of keratin in natural history museums, but also the data can be used to adjust museum storage conditions to ensure maximum specimen lifetime and utility.

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LITERATURE CITED

Arai, K., T. Hirata, S. Nishimura, M. Hirano, and S. Naito. 1993. Cross-linking structure of keratin. 4. The number of cross-linkages in low-sulfur components and the volume fraction of high-sulfur domains in various alpha-keratin fibers. Journal of Applied Polymer Science 47:1973–1981.

Benson, J.R. and P.E. Hare. 1975. O-phthalaldehyde: Fluorogenic detection of primary amines in the picomole range: Comparison with fluorescamine and ninhydrin. Proceedings of the National Academy of Sciences, USA 72:612–622.

Bonser, R.H.C. and P.P. Purslow. 1995. The Young's modulus of feather keratin. Journal of Experimental Biology 198:1029–1033.

Bradbury, J.H. 1973. The structure and chemistry of keratin fibers. Advances in Protein Chemistry 27:111–211.

Crewther, W.G., R.D.B. Fraser, F.G. Lenox, and H. Lindley. 1965. The chemistry of keratins. Advances in Protein Chemistry 20:191–346.

Darkus, R.L. and J.M. Gillespie. 1971. Breed and species differences in the proteins of four genera of Caprini. Australian Journal of Biological Science 24:515–524.

Dowling, L.M. and L.G. Sparrow. 1991. Sequences of wool keratin proteins: The CSIRO connection. Trends in Biochemical Science 16:115–118.

Gillespie, J.M. and M.J. Frenkel. 1974. The diversity of keratins. Comparative Biochemistry and Biophysics 4713:339–346.

Gillespie, J.M. and R.C. Marshall. 1977. Proteins of the hard keratins of *Echidna,* hedgehog, rabbit, ox and man. Australian Journal of Biological Sciences 30:401–409.

Hicks, J.W. 1977. *Microscopy of Hairs.* Federal Bureau of Investigation, Washington, DC. 53 pp.

Hrdy, D. and H.P. Baden. 1973. Biochemical variation of hair keratins in man and non-human primates. American Journal of Physical Anthropology 39:19–24.

James, V.J., K.E. Wilk, J.F. McConnell, E.P. Baranov, and Y. Amemia. 1995. Intermediate filament structure of baboon hair. International Journal of Biological Macromolecules 17:99–104.

Means, G.E. and R.E. Feeney. 1971. *Chemical Modification of Proteins.* Holden-Day, Inc., San Francisco, California. 254 pp.

Menefee, E. 1977. Physical and chemical consequences of crosslinking, with application to the determination of crosslink density. Advances in Experimental Medical Biology 86A:307–327.

Mercer, E.H. 1961. *Keratin and Keratinization: An Essay in Molecular Biology.* Pergamon Press, New York, New York. 314 pp.

Parry, D.A.D., W.G. Crewther, R.D.B. Fraser, and T.P. MacRae. 1977. Structure of alpha-keratin: Structural implication of the amino acid sequences of the type I and type II chain segments. Journal of Molecular Biology 113:449–454.

Steinert, P.M., A.C.T. North, and D.A.D. Parry. 1994. Structural features of keratin intermediate filaments. Journal of Investigative Dermatology 103:19s–24s.

Tucker, D.J., A.H.F. Hudson, A. Laudani, R.C. Marshall, and D.E. Rivett. 1989. Variation in goat fibre proteins. Australian Journal of Agricultural Research 40:675–683.

von Endt, D.W. 1994. Spirit collections: A preliminary analysis of some organic materials found in the storage fluids of mammals. Collection Forum 10:10–19.

Whitaker, J.R. and M. Fujimaki. 1980. *Chemical Deterioration of Proteins.* American Chemical Society, Washington, DC. 268 pp.

Wilk, K.E., V.J. James, and Y. Amemiya. 1995. The intermediate structure of human hair. Biochimica et Biophysica Acta 1245:392–396.

Wortman, F.J. and H. Zahn. 1994. The stress/strain curve of alpha-keratin and the structure of the intermediate filament. Textile Research Journal 64:737–743.

Yu, J., D.-W. Yu, D.M. Checkla, I.M. Freedberg, and A.P. Bertolino. 1993. Human hair keratins. Journal of Investigative Dermatology 101:565–595.

THE INTERACTIONS OF PRESERVATIVE FLUID, SPECIMEN CONTAINER, AND SEALANT IN A FLUID COLLECTION

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Abstract.—The Leiden Anatomy Museum houses a collection of human body preparations made over a period of 400 yr. The anatomical and pathological collections from the 18th and 19th century include preparations by famous anatomists such as Albinus and Sandifort. Over the last 4 centuries there have been evolutionary changes in conservation methods. Some significant problems caused by the use of different combinations of preservative fluids, jars, and sealants are discussed with respect to how they relate to the physical processes that take place in fluid-filled, sealed containers. One often-overlooked problem is negative pressure in Plexiglas® jars, which causes warping and the occasional implosion of the container. This problem can be solved by the use of a bidirectional valve.

The Anatomy Museum of Leiden University has a collection of human specimens that have accumulated since the Anatomical Theater was founded in the 1590s. The extensive collection, which covers anatomy, embryology, teratology, pathology, and physical anthropology, is an important teaching aid in medical education today.

The oldest fluid collections are from the 18th century and were obtained from anatomists including Rau, Albinus, Sandifort, and van Doeveren. In the 19th century, the holdings were further extended by the donation or purchase of the collections of Rocquette, Brugmans, Bonn, Broers, and Suringar. These collections are still consulted regularly by foreign scientists, because they contain unique specimens with their original documentation. Almost all preparations from this period were preserved in "spirit of wine," now known as ethanol (Down 1989, Elshout 1952). In the Sandifort collection, turpentine was also used (Elshout 1952) to clear the tissue to show the injected vascular system. The specimens were stored in hand-blown cylindrical glass jars with a flanged top to accommodate a seal. The jars were closed with a cork stopper or a plate of schist, which was sealed with a wax-based material. To prevent loosening of the lids, a wet pig's or sheep's bladder was stretched over the top of the jar and secured with a thin string.

At the end of the 19th century, the whole process of fixation and preservation of organic tissue was influenced by the introduction of formalin (Blum 1893). Kaiserling (1896) discovered procedures to preserve color in formalin-fixed specimens. At this time, jars became more sophisticated and were also made in rectangular or square shapes. With the modernization of the jars, glass lids were introduced, which were sealed with a mixture of paraffin, masticated rubber, sheep fat, and lead plaster (lead salt of fatty acids).

After the Second World War, the chemical industry produced many new synthetic sealing materials. In the 1960s, newly prepared specimens were placed in rectangular jars made from a Plexiglas® plate. The great advantage of these containers was the lack of visual distortion compared to the cast rectangular glass

containers. Also, these jars could be made in every size. However, in the course of time, Plexiglas® jars warp inwardly and the glued joints fail.

In the 1970s, universal transparent silicone rubber was introduced as a sealant in several Dutch institutes. Because ethanol (EtOH) affects silicone rubber (Reilly 1989), about 10 yr ago we began to use Dicera 4799, a wax-based sealant developed for the spirit collections of the National Natural History Museum of the Netherlands. This sealant is not affected by EtOH; however, with a significant decrease in storage temperature the glass lids can crack (Simmons 1995). We noticed in the formalin collection that with time several large rectangular glass jars, sealed with silicone rubber spontaneously cracked. Because of the irreversibility of silicone rubber, 5 yr ago, Tixophalte®, a cold-hardening bitumen, began to be used as an alternative. This sealant has low permeability, ethanol or formalin does not affect it, and it is soluble in "white gas," a petroleum distillate with a high boiling point (100–140°C). Its adhesion to smooth glass is very poor but to ground glass is fair. While in use it was noticed that, with wide fluctuations in storage temperature, many lids loosened.

The museum also manages the research collections of the Department of Anatomy and Embryology. In contrast to the museum collection, the research collections have to be easily accessible. Therefore these specimens are stored in containers with removable lids. The oldest containers (manufactured around 1950) were cylindrical glass jars with Bakelite® screw tops. In agreement with Simmons (1995), we noticed that in a period of 10–20 yr the Bakelite® lids of jars containing formalin became brittle and cracked. To slow down this deterioration and to provide a tighter seal Parafilm M® could be placed between the lid and the jar (Reilly 1989).

Around 1970, low-priced, thin-walled polyethylene (PE) and polypropylene (PP) buckets with snap-on lids were also used. In the ethanol collections, problems such as hardening and splitting of the plastics and a consequent rapid loss of preservative fluid were encountered. In the last 5 yr, we replaced these buckets with thick-walled high-density polyethylene (HDPE) containers with screw lids of the same material. Loss of preservative fluid is slower, but still occurs.

In both ethanol- and formalin-preserved collections, the combination of preservative fluid, jar, and sealant influences the quality of specimen conservation. Too many collections suffer from problems such as a rapid loss of preservative fluid, warping or cracking of jars and lids, and loosening of the lids.

THEORETICAL CONSIDERATIONS

To relate problems such as loss of preservative fluid, loose lids, cracked glass, and warped Plexiglas® to the physical interaction between the preservative fluid, the jar, and the sealant, the physical processes that take place in a fluid-filled sealed container are considered.

Temperature and pressure.—Horie (1994) described the relationship between temperature and pressure in fluid-filled, sealed jars. As a result of changes in temperature, the internal pressure in a sealed jar fluctuates. The internal pressure depends on the vapor pressure of the fluid and the different thermal expansion rates of the jar, the fluid, and the air above the fluid.

Thermal expansion rates can be expressed by the volumetric expansion coefficient (α), which is the ratio of the change in volume per degree Centigrade to

Table 1. Volumetric expansion coefficients at 20°C for different fluids, different types of glass, and different polymers used in fluid preservation.

Substance	α_{20} (10^{-6} °C^{-1})	Reference
Water	207	Hodgman (1957)
Ethanol, 50%	830	Hodgman (1957)
Ethanol, 99.3%	1,120	Hodgman (1957)
Glycerol	505	Hodgman (1957)
Borosilicate glass	27	Hodgman (1957)
"Commercial glass"	32	Hodgman (1957)
Polymethylmetacrylate (Plexiglas®)	219	Eriks Company (1991–1992)
Polypropylene	540	Eriks Company (1991–1992)
Polyethylene (low and high density)	600	Eriks Company (1991–1992)

the volume at 0°C. The value of the coefficient varies with the temperature (Weast 1970–1971). For example, at 20°C EtOH has an expansion coefficient, which is approximately 40 times higher than borosilicate glass, whereas that of water is eight times higher than glass (Table 1). For both fluids, an increase in temperature results in a rise of the fluid level in the jar and, consequently, compression of the air above it. Combined with the increased vapor pressure, the seal will be under considerable stress and the lid can loosen.

The ratio of the volume of the fluid and the remaining air volume in the jar also influences the pressure that builds up when the temperature increases (Horie 1994). This is illustrated by the following example. A 100-ml glass jar is filled with EtOH up to 95 ml. An increase in temperature from 15–25°C results in a volume increase up to approximately 96 ml. Consequently, the remaining air volume is reduced from 5 ml to 4 ml, which means a 20% compression of the original air volume (Fig. 1). When the same jar is filled with 98 ml of fluid, the same increase in temperature will give a new volume of approximately 99 ml. In this case, the remaining air volume is reduced from 2 ml to 1 ml, in effect a 50% compression of its volume (Fig. 2). This means that the higher the jar is filled with fluid, the greater the compression of the air volume, and the greater the internal pressure. A temperature decrease results in the opposite effect and creates a negative pressure in the glass container, which may result in the cracking of the glass lid or jar.
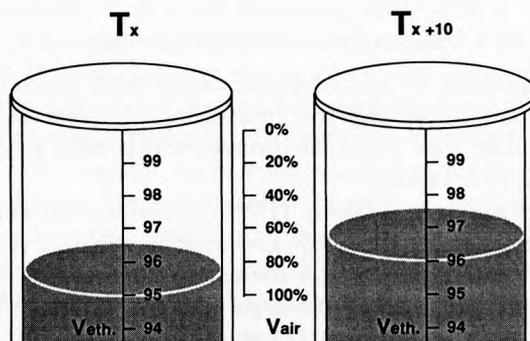

Figure 1. Sealed glass jar filled with EtOH up to 95 ml before and after a 10°C increase in temperature.
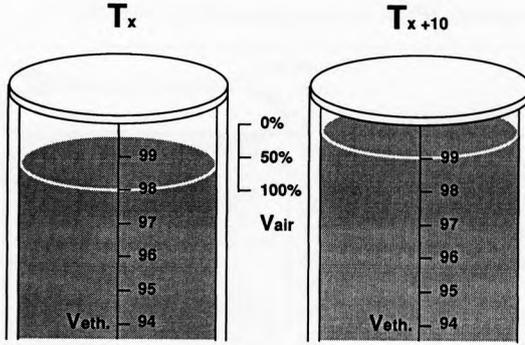
$$T_x \qquad\qquad T_{x+10}$$

Figure 2. Sealed glass jar filled with EtOH up to 98 ml before and after a 10°C increase in temperature.

Diffusion and permeability.—Brokerhof (1993) concluded in her survey on conservation problems in natural history collections in The Netherlands that with respect to the fluid preserved collections, the loss of preservative fluid in the jars is one of the major problems in collection maintenance. When there are no leaks, the fluid loss can be attributed to the physical process of diffusion of the fluid through the container, lid, and seal. The diffusion rate depends on the permeability of the jar, the lid, and the seal. It also depends on the temperature. The higher the permeability of the components used and the higher the temperature, the faster this process takes place.

Yasuda and Stannett (1975) described the rate of permeation through a polymer film as expressed by parameters that are characteristic of the polymer. One parameter is the permeability coefficient, P:

$$P = \frac{(\text{amount of permeant})(\text{film thickness})}{(\text{area})(\text{time})(\text{pressure drop across the film})}$$

When a permeant does not interact with the polymer, P is a constant that is characteristic for the permeant-polymer system, which is the case for the permeation of gases, such as O_2 and N_2, through most polymers (Table 2). This means that when the film thickness is increased by a factor two, the amount of gas permeating through the film will be two times smaller. However, when liquids and vapors permeate through polymers, they interact, and P is no longer constant. Therefore, the transmission rate, Q, is often used for practical purposes when the pressure drop across the film equals that of the saturated vapor pressure:

$$Q = (\text{amount of permeant})(\text{film thickness})/(\text{area})(\text{time}).$$

Though Q is not truly constant, it can be used for comparing orders of magnitude (Table 3). One exception is the permeation of water through bitumen. Because there is no interaction between water and bitumen, P is characteristic for the permeant-polymer system. This means that the water diffuses as a gas following Fick's Diffusion Law (Anderson and Wright 1941):

$$W = KAPt/L,$$

where: W = amount of water diffused (g), L = thickness of film (cm), A = area

Table 2.   Permeability constants (P) of different polymers for oxygen.

Polymer	Temperature (°C)	P	Reference
Bitumen (asphalt)	25	0.73	Anderson and Wright (1941)
Polychloroprene (neoprene)	25	263	Yasuda (1966)
Polydialkylsiloxane (silicone rubber)	30	4,599	Yasuda (1966)
Polyisoprene (natural rubber)	25	1,564	Yasuda (1966)
Polyethylmetacrylate (Plexiglas®)	25	76	Yasuda and Stannett (1975)
Polypropylene	30	151	Yasuda (1966)
Polyethylene, low density	30	260	Yasuda (1966)
Polyethylene, high density	30	34	Yasuda (1966)
Polyethylene terephthalate (Mylar®)	30	3.0	Yasuda (1966)
Polyvinylidene chloride	30	0.35	Yasuda (1966)

$P = cm^3\ O_2 \times mm$ thickness $\times m^{-2}$ area $\times 24\ hr^{-1} \times bar^{-1}$.

of film (cm^2), P = vapor pressure differential (mm Hg), t = time of diffusion (hr), and K = permeability coefficient (g/cm mm Hg hr).

With this equation it is possible to calculate the loss of water in a glass jar sealed with bitumen. The permeability coefficient for water for a bituminous sealant like Tixophalte® is approximately 10^{-8} g/cm mm Hg hr (Shell Company, product information). The following example will illustrate the influence of the sealant on the diffusion rate. Consider a water-filled cylindrical glass jar with a 5-mm rim and an inner diameter of 10 cm. The thickness of the sealant between the rim and top plate is 2 mm (Fig. 3) Assuming that glass is impermeable to water, 50% relative humidity outside the jar, and a storage temperature of 20°C, the loss of water is 0.0099 g/yr for Tixophalte®. The very low value of approximately a 1-ml loss of water in a century makes Tixophalte® an almost ideal sealant with respect to permeability.

In addition to the permeation of the preservative fluid through the container materials, the permeation of oxygen should also be taken in consideration. Stoddart (1989) described the importance of keeping the amount of free oxygen inside the jar as low as possible. Oxidation of formaldehyde may cause a drop in the pH (acidification) of the preservative fluid. Oxidation may also affect lipids, amino acids, and pigments. Therefore, container materials should have low permeability to oxygen.

In Tables 2, 3 the permeability coefficients for oxygen and the transmission rates for water for different polymers are listed. Many of these polymers are used as container materials or as sealants in fluid preservation. Note that a polymer

Table 3.   Transmission rates of different polymers for water (Yasuda 1966).

Polymer	Temperature (°C)	Q
Polychloroprene (neoprene)	39.5	6.1
Polyisoprene (natural rubber)	39.5	5.9
Polymethylmetacrylate (Plexiglas®)	39.5	14
Polyethylene, low density	39.5	0.43
Polyvinylidene chloride	39.5	0.12

$Q = g\ H_2O \times mm$ thickness $\times m^{-2}$ area $\times 24\ hr^{-1}$.
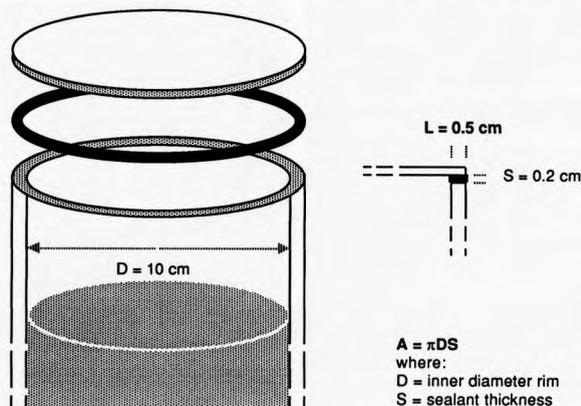
Figure 3.   Practical example of a sealed glass jar filled with water used to calculate the amount of water diffused in a period of 10 yr with Fick's Diffusion Law. The thickness and area of the seal, the pressure differential between the internal and external water vapor pressure, and the time of diffusion are all linearly related to the calculated result. For example, when the seal thickness (determined by the distance between inner and outer rim of the jar) is twice as great, diffusion will be twice as slow.

with a high P value for oxygen has a higher permeability for this gas than a polymer with a lower P value. In the case of the permeation of water, the same relationship can be applied to Q. Finally, Table 4 lists values for water vapor and oxygen permeating through different polymer foils under standard conditions.

OBSERVATIONS

The variety of preservative fluids, jars, and sealants used in our collections gave us the opportunity to determine whether the problems related to them could be attributed to the physical interaction between the different components used.

We recently carried out a survey of the different combinations of preservative fluid, jar, and sealant that have been used until now and related them to the most significant problems observed in the last 10 yr (Table 5). To retrieve valid data from the collections, this survey was limited to the museum collection, because it has been managed and maintained by a single person during the last 13 yr.

Historical collection.—Eighty percent EtOH has been used as preservative fluid in cylindrical glass containers sealed either with Dicera 4799 (a wax-rubber mixture that has to be melted), or Tixophalte® (a cold-hardening bitumen). Both

Table 4.   Permeability of different polymer foils to oxygen (DIN 53380) and to water vapor (DIN 53122). The data are from Domininghaus (1988).

Polymer	Temperature (°C)	Thickness (μm)	Water (g m^{-2} 24 hr^{-1})	Oxygen (cm^3 m^{-2} 24 hr^{-1} bar^{-1})
Polypropylene	25	40	2.1	1,900
Polyethylene, low density	23	100	1.0	2,000
Polyethylene, high density	25	40	0.9	1,890
Polyethyleneterephthalate	23	25	0.6	80/110
Polyvinylidene chloride	25	25	0.1/0.2	1.7/11

Table 5. The different preservative fluids, jars, and sealants related to the most significant problems observed in the collections. All combinations are stored in rooms without environmental control. With regard to the temperature, daily fluctuations of 10°C are not exceptional. Because of the variety in sizes within the same category of lid type and jar type, the rate of fluid loss is just a rough indication.

| Collection	Preservative fluid	Jar type	Lid type	Sealant	Period of use (yr)	Rate of fluid loss	Problems	Time before problems noted after sealing (yr)
Historical (18th–19th century)	EtOH 80%	cylindrical glass	glass plate	Dicera 4799	10	very low	cracked lids	0–1
Modern anatomy	formalin 4%	cylindrical glass	glass plate	Tixophalte®	5	very low	loose lids	0–1
		rectangular glass	glass plate	silicone rubber	20	low	cracked jars	0–20
		rectangular glass	glass plate	Tixophalte®	5	very low	loose lids	0–1
		rectangular Plexiglas®	Plexiglas® plate	Plexiglas® cement	20	high	warped jars, loose joints	10–20
Modern pathology	glycerin 65%	rectangular Plexiglas®	Plexiglas® plate	Plexiglas® cement	30	medium	warped jars, loose joints	20–30
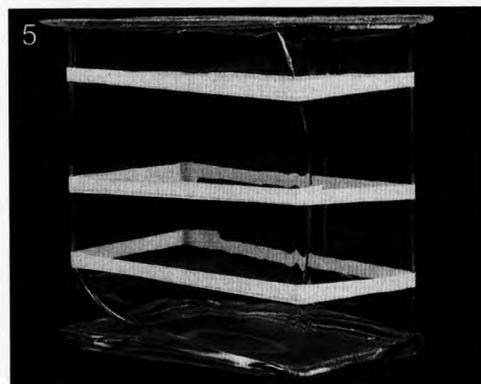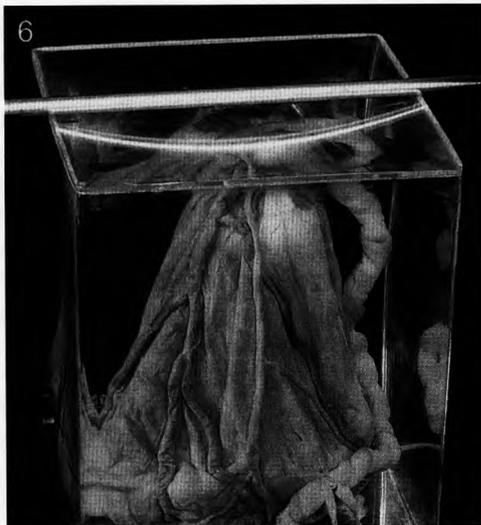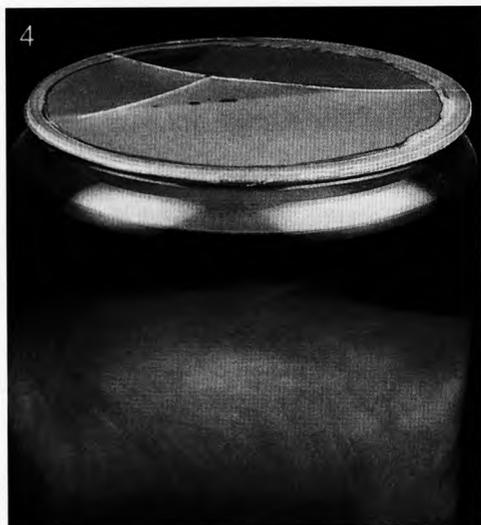
Figure 4.   Cylindrical glass jar with cracked glass lid.

Figure 5.   Cracked rectangular glass jar with a glass lid.

Figure 6.   Warped Plexiglas® jar. The reflection of the metal rod shows the extent of warping of the upper side.

Figure 7.   Partially split seam showing the small cracks in the seam between a Plexiglas® jar and a Plexiglas® lid.

sealants are unaffected by EtOH and have low permeability. Fluid levels are stable when seals are intact. In the case of Tixophalte®, the loosening of the glass lids of several jars was noticed within 1 yr after sealing and occurred most frequently in summer, when there was a significant increase in storage temperature. In the case of Dicera, the glass plates of several large-diameter jars cracked (Fig. 4), especially when the jars were moved to the storage room in the basement, where the temperature is significantly lower (>5°C) than the place were they were filled and sealed.

Modern anatomy collection.—This collection is primarily preserved in 4% formalin in rectangular glass or Plexiglas® jars. The glass jars, which were sealed with universal transparent silicone rubber, showed a slight decline in fluid level

over 15 yr. Occasionally, large jars (>5 L) crack spontaneously (Fig. 5). It was observed that most of the cracked jars were the old, flanged, rectangular jars, manufactured at the beginning of this century. In the last 5 yr, a small number of containers in the collection have been sealed with Tixophalte®. As with the historical collection, the loosening of several glass lids was recorded within 1 yr after sealing. It was also observed that Plexiglas™ jars, filled with 4% formalin, warped inwardly during the period of observation (Fig. 6). Although the Plexiglas™ containers show no visual decline in fluid level, it drops significantly (5–10%) when the container is vented and the internal pressures are released. Also, the glue joints show little cracks that propagate from the outside edge inward (Fig. 7). These are indications that the sides of the jar have been under considerable stress.

Modern pathology collection.—The Plexiglas™ jars filled with 65% glycerin show the same problems as the modern anatomy collection after 20–30 yr. Several jars were leaking, and within the last 5 yr, several jars burst at the joints spilling the fluid in a matter of seconds.

DISCUSSION

Problems related to temperature.—Horie (1994) and Simmons (1995) reported that temperature fluctuations in the storage environment can cause cracking and loosening of glass lids. In the historical collection these are the main problems encountered in the first year after sealing (Table 5). Because of the large daily temperature fluctuations in our storage facilities (>10°C) and the large difference in expansion coefficients between ethanol and glass (Table 1), it can be assumed that pressure fluctuations inside the jar are high. Consequently, the glass lids will be under considerable stress, which can lead to them cracking or loosening. However, this does not explain why most of the lids of jars sealed with Dicera 4799 crack, whereas most of the lids sealed with Tixophalte® loosen.

The explanation for this can be found in the different methods of applying the sealant. Dicera is applied by pre-heating the glass lid (whereby the product melts between the rim and the lid of the jar) to get a tight and leak-free seal (van Dam 1996). Before Dicera cools and solidifies, the air inside the jar expands as a result of the heat of the lid and escapes through the seal to the outside environment to approximate an equilibrium between the inside and outside pressures. Once the lid cools down, the sealant solidifies and the air flux to the outside of the jar stops. The air underneath the lid also cools and, because pressure is linearly related to temperature, this results in a decrease of internal pressure. Consequently, there will be a slight negative pressure inside the jar. If the storage temperature increases, the absolute pressure in the jar will increase but still be lower than in a similarly stored jar sealed with a cold-hardening product such as Tixophalte®. If the storage temperature decreases, the negative pressure caused by the thermal contraction of the jar materials and its contents will increase the existing negative pressure. The cracking of the lids was noticed principally in our basement storage room, where the temperature fluctuates around a lower average temperature than where the jars are filled and sealed. Furthermore, it was noticed that the problem only occurred in large diameter (>15 cm) jars. Although these jars are highly pressure resistant because of their cylindrical shape and oval bottom, the lids,

made of straight 3-mm thick ground glass, are less resistant and therefore more prone to cracking.

No heat is used to apply Tixophalte® (van Dam 1996), but weights are put on the lid until the sealant has hardened. In this case, the internal pressure is then slightly higher than the outside pressure because of compression of the air pocket while the lid was being affixed. With an increase in temperature this pressure difference will only rise more, which can easily result in the loosening of the lid. Because Tixophalte® has a low tear strength and low adhesion to glass compared to a product such as (universal) transparent silicone rubber, loosening of the lid can be expected, especially in summer.

In the modern anatomy collection the same problem was encountered with the formalin-filled rectangular glass jars sealed with Tixophalte®. Formalin has a smaller expansion coefficient than EtOH, but it is larger than that of glass (Table 1). Therefore, the loosening of lids can be attributed to the same causes as in the historical collection.

In the modern anatomy collection some of the large, old, flanged-type rectangular jars sealed with silicone rubber spontaneously cracked resulting from temperature fluctuations. Because of the excellent adhesion of silicone rubber to glass, together with its high tear strength, an increase in temperature will not push off the lid, nor will a temperature decrease break the seal. In addition, a rectangular jar is less pressure resistant than its cylindrical counterpart. Especially old, more brittle glass is likely to crack under pressure. In this case, the glass lid and the seal seem to be more stress resistant than the jar. Despite their fragility, these jars are preferred to cylindrical jars because of their limited visual distortion, which is essential for demonstration in medical teaching.

In the 18th century, similar problems caused by fluctuations in environmental temperature must have occurred. This could explain the use of pig's or sheep's bladders to prevent the loosening of lids. Furthermore, the cylindrical shape combined with a thick lid made of schist is presumed to be less prone to cracking. This calls to question whether these old techniques might be more durable than many modern techniques.

Problems related to diffusion.—The physical process of diffusion of either the preservative fluid or oxygen through a sealed specimen container affects the preservation quality of the specimens. The diffusion of preservative fluid through the container results in loss of preservative fluid, changes in the preservation properties of the fluid, fungal attack or shrinkage, and drying out of the specimen (Reilly 1989). The diffusion of oxygen into the container accelerates the oxidation processes inside the jar, which can result in acidification of the preservative fluid, breakdown of lipids, loss of color, etc. (Stoddart 1989).

Glass is practically impermeable to most chemicals and durable, and therefore it is the preferred container material for fluid preservation. When using glass jars with glass lids, the diffusion rate only depends on the type of sealant that is applied.

In the historical collection, the permeation of EtOH through Dicera 4799 and Tixophalte® appears to be very low (Table 5). In fact, no drop in fluid level was observed. Anderson and Wright (1941) reported the very low permeability of bituminous materials such as Tixophalte® to water and oxygen. Dicera 4799 is a mixture of polyisobutylene rubber and refined hydrocarbon waxes, and in fact,

like bitumen, it is largely composed of hydrocarbons. Both materials are very inert substances and are not affected by EtOH.

The glass jars in the modern anatomy collection, which are sealed with silicone rubber (from different manufacturers), show a more rapid loss of preservative fluid than the jars sealed with Tixophalte® (Table 5). Silicone rubber appears to be more permeable to formalin than Tixophalte®. A comparison of the permeability constants for oxygen for different rubbers shows that silicone rubber ranks highest in permeability, whereas bitumen has the lowest value (Table 2). For this reason, silicone rubber seems to be less suitable as a sealant.

In polyethylene, polypropylene, and Plexiglas® jars, the whole surface is permeable to most preservative fluids, thus these containers have a larger area for diffusion compared to glass jars, which results in a more rapid loss of preservative fluid. Considering the water permeability of plastics (Tables 3, 4), the transmission rates of polypropylene (PP), low-density polyethylene (LDPE), and high-density polyethylene (HDPE) are all acceptable values. Polyethylene terephthalate (PET) and polyvinylidene chloride (PVDC) show even lower transmission rates. However, the transmission rate of Plexiglas®, when compared to LDPE, is relatively high. This explains quite well the more rapid loss of preservative fluid in Plexiglas® containers used in the modern anatomy and pathology collections (Table 5). The difference in the rate of fluid loss in these two collections is the result of the different preservative fluids used. Four-percent formalin mainly consists of water, whereas 65% glycerin mainly consists of glycerol. The larger glycerol molecules will permeate through Plexiglas® at a much slower rate (or probably not at all).

There are also great differences in the oxygen permeability of various plastics. When comparing PP, LDPE, HDPE, and polyethylmetacrylate (PEMA), an acrylic similar to Plexiglas® (polymethylmetacrylate, PMMA), HDPE has the lowest permeability (Table 2). However, the permeability value for PET is significantly lower than that of HDPE (Table 4), whereas the value for PVDC is extremely low, like that of bitumen (Tables 2, 4). For this reason, PVDC-containing laminates are commonly used as vacuum sealing foils in the food industry. Despite the high permeation of oxygen through materials such as PP, PE, and Plexiglas®, they are frequently used as jar or lid and liner materials in fluid preservation.

Problems related to Plexiglas®.—Reilly (1989) described the warping of Plexiglas® containers in spirit collections. As a remedy, she suggested changing EtOH for formalin. In our modern anatomy and pathology collections preserved in formalin and glycerin respectively, the same problems were encountered (Table 5). However, the warping of Plexiglas® containers filled with formalin progressed faster than those filled with glycerin. This phenomenon is not yet completely understood but is being further investigated in cooperation with the Leiden Institute of Chemistry of Leiden University.

Although it is not known whether the negative internal pressure (most apparent when aerating the jars) is the only factor responsible for the warping of Plexiglas®, it should be prevented. Venting the jars periodically can do this, which is possible by drilling a refill hole and closing the hole with a removable stopper. This venting by hand intensifies the maintenance routine considerably. Therefore, a far better solution is the use of a valve that vents the jar automatically.

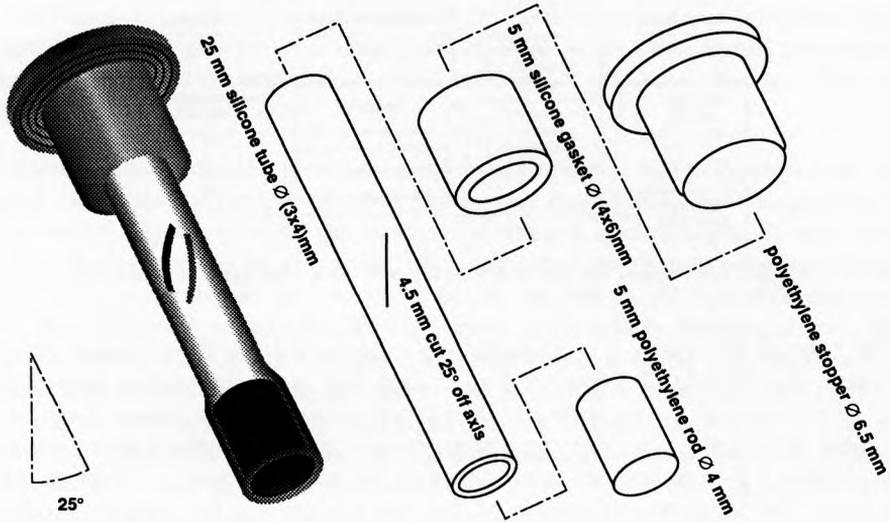*Bidirectional valve.*—We developed a bidirectional valve that prevents the neg-

Figure 8.   Bidirectional valve (design by M.J. Aarents and A.J. van Dam).

ative pressure in Plexiglas jars and ameliorates the high pressure fluctuations caused by temperature changes. It was put into service 2 yr ago and has functioned properly thus far. The valve is constructed of a polyethylene stopper, a silicon gasket, and a polyethylene rod (Fig. 8) and is inserted through a hole in the lid (Fig. 9).

It is suitable for all water-based preservative fluids such as formalin, phenoxyethanol, and Kaiserling solution. Its use in combination with ethanol is still under investigation. The cost of the materials is less than 5 cents (US) per valve.
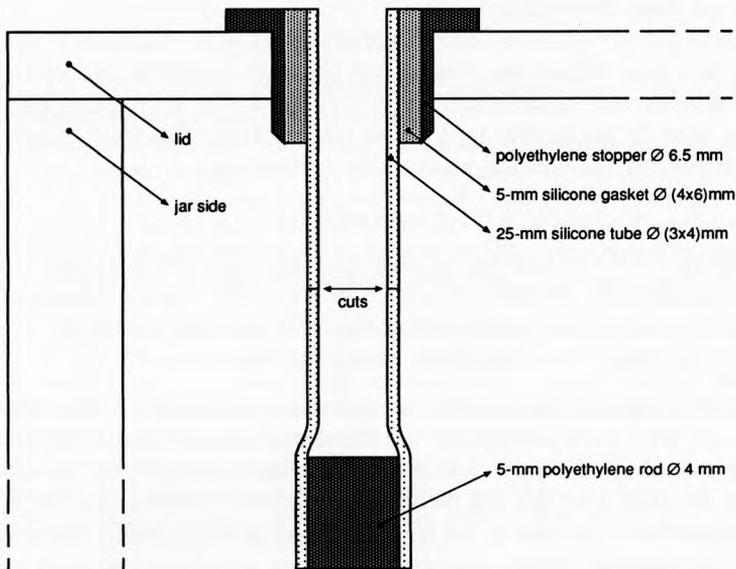
Figure 9.   Cross-section of a bidirectional valve *in situ.*
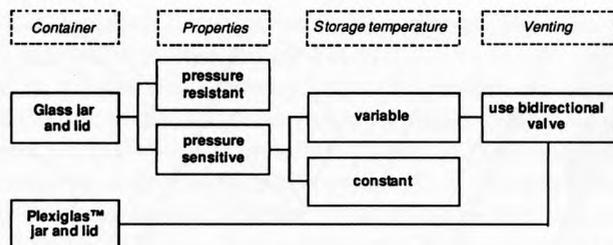
Figure 10. Factors determining the use of a bidirectional valve with glass or Plexiglas℗ jars.

The core of the valve is a 25-mm-long silicone tube with an inside diameter of 3 mm and outside diameter of 4 mm. One end is closed with a polyethylene plug. The other end is connected by a silicone gasket, which placed over the 25-mm tube, to a hollow polyethylene stopper, which has had the bottom removed. Two oblique 4.5-mm cuts are made through the gasket, about 25 degrees off the long axis. The location and length of the cuts are critical for proper functioning of the bidirectional valve. However, tolerances of up to 10% are allowed. Massaging silicone grease in the cuts prevents fusing of the rubber at their cut surfaces.

When the valve is installed on a sealed jar and a slight negative pressure is created, the silicone gasket expands and the valve opens immediately. The inlet of air stops when the pressure in the jar almost equals the outside pressure. When the valve is open, vapor does not escape because air is sucked in. With increasing internal pressure, the silicone gasket is compressed and opens when a critical pressure is reached. Vapor will escape until the internal pressure is equal to a critical pressure. The pressure differential between the external and internal pressures for this critical point is between 0.1 and 0.15 bar. This means that the valve does not react to minor increases in temperature. This prevents excessive evaporation of the fluid through the valve.

This bidirectional valve can be effectively used with Plexiglas℗ jars and glass jars with glass lids, which are considered pressure sensitive and stored in rooms with large temperature fluctuations (Fig. 10). However, a constant temperature in the storage area is preferable to the use of a valve, otherwise, glass containers lose a great part of their function as oxygen barriers.

CONCLUSIONS

In fluid-preserved collections, storage systems with low permeability to fluids, vapors, and gasses are the best choice. In practice this means the use of glass jars with glass lids and a low permeance sealant. To prevent loosening of the lids or cracking of the glass, two conditions should be met.

1. Store containers at a constant temperature, preferably a few degrees lower than the place where the containers are filled and sealed (Horie 1994). The slight negative pressure in the jar caused by the small decrease in temperature will prevent the loosening of the lid. Sealing procedures wherein the lid is preheated present difficulties: it is best if the treatment and storage-facility temperatures are equivalent. In addition, high-temperature storage should be avoided to slow bio-deterioration.

2. Fill each container to a maximum of 90% of its volume with ethanol-based preservative fluids and up to a maximum of 95% with water-based preservative fluids. This reduces the amount of stress on the lid, seal, and jar caused by temperature fluctuations.

Containers incorporating plastics, such as polyethylene and polypropylene, have a higher permeability for preservative fluids than their glass counterparts, but can still be useful. However, their permeation by oxygen is very high. To slow down the oxidation processes in glass jars with polyethylene or polypropylene lids, it is worthwhile to consider the use of bitumen- or polyvinylidene-chloride-coated liners. The diffusion of oxygen through these materials is extremely low. With respect to stress, a constant storage temperature seems to be less important because of the flexibility of the jar and/or the lid. However, Simmons (1995) reported that screw-on jar lids made of rigid plastics tend to "back off" or unscrew.

By using a bidirectional valve one can overcome the risk of cracking glass jars or lids resulting from lack of temperature control, as well as the risk of irreversible damage to Plexiglas® jars caused by negative pressure. However, it remains to be determined whether this negative pressure is the only factor responsible for the warping of Plexiglas®.

Clearly, the physical interactions among preservative fluids, jars, and sealants have a direct bearing on collection maintenance. Therefore, materials should be selected after a careful consideration of all parameters of storage are taken into account.

Literature Cited

Anderson, A.P. and K.A. Wright. 1941. Permeability and absorption properties of bituminous coatings. Industrial and Engineering Chemistry 33(8):991–995.

Blum, F. 1893. Der Formaldehyd als Antisepticum. Münchener medizinische Wochenschrift 40(32): 601–602. [Formaldehyde as antiseptic. Munich Medical Journal, in German].

Brokerhof, A.W. 1993. *De Conservering van Natuurhistorische Collecties: Een Inventarisatie van de Problemen, Bestaande Kennis, Kennisleemtes en Behoefte aan Onderzoek.* Central Research Laboratory for Objects of Art and Science, Amsterdam. 84 pp. [*The Conservation of Natural History Collections: A Survey of the Problems, Existing Knowledge, Knowledge Gaps, and Need for Research,* in Dutch].

Domininghaus, H. 1988. *Die Kunststoffe und ihre Eigenschaften.* VDI Verlag, Düsseldorf. 905 pp. [*Polymers and their Properties,* in German].

Down, R. 1989. "Old" preservative methods. Pp. 33–38 in *Conservation of Natural History Specimens: Spirit Collections* (C.V. Horie, ed.). Manchester Museum and Department of Environmental Biology, University of Manchester. 114 pp.

Elshout, A.M. 1952. *Het Leidse Kabinet der Anatomie uit de Achttiende Eeuw: De Betekenis van een Wetenschappelijke Collectie als Cultuurhistorisch Monument.* University Press of Leiden, Leiden. 124 pp. [*The Leiden Anatomical Cabinet of the 18ᵗʰ Century: The Significance of a Scientific Collection as a Cultural Historic Monument,* in Dutch].

Eriks Company. 1991–1992. *Technische Kunststoffen.* Eriks Company, Alkmaar, Netherlands. 64 pp. [*Technical Polymers,* in Dutch].

Hodgman, M.S. (ed.). 1957. *Handbook of Chemistry and Physics,* 39th ed. Chemical Rubber Company, Cleveland, Ohio. 3,213 pp.

Horie, C.V. 1994. Environmental control for spirit specimens. Biological Curators Group Newsletter 6(4):43–44.

Kaiserling C. 1896. Über die Conservirung von Sammlungspräparaten mit Erhaltung der natürlichen Farben. Berliner Klinische Wochenschrift. 33(35):775–777. [About the preservation of collection specimens to maintain the natural colors. Berlin Clinical Journal, in German].

Reilly, M. 1989. Curation of a small collection. Pp. 95–112 in *Conservation of Natural History Specimens: Spirit Collections* (C.V. Horie, ed.). Manchester Museum and Department of Environmental Biology, University of Manchester, 114 pp.

Shell Company. *Mexphalte: De Absolute Winnaar onder de Bitumen.* Shell Company, Rotterdam. 12 pp. [*Mexphalte, the Absolute Winner amongst Bitumen,* in Dutch].

Simmons, J.E. 1995. Storage in fluid preservatives. Pp. 161–186 in *Storage of Natural History Collections: A Preventive Conservation Approach* (C.L. Rose, C.A. Hawks, and H.H. Genoways, eds.). Society for the Preservation of Natural History Collections. 448 pp.

Stoddart, R.W. 1989. Fixatives and preservatives: Their effects on tissue. Pp. 1–26 in *Conservation of Natural History Specimens: Spirit Collections* (C.V. Horie, ed.). Manchester Museum and Department of Environmental Biology, University of Manchester. 114 pp.

van Dam, A.J. 1996. Vloeistofpreparaten. Pp. B1–B15 in *Conservering Natuurhistorische Collecties* (A.W. Brokerhof, J. Boerhof, J. Fokkema, and R. Schouten, eds.). Working Group Natural History Collections of the Section Natural History Museums of the Dutch Museum Society, Amsterdam, Netherlands. 140 pp. [*Conservation of Natural History Collections,* in Dutch].

Weast, R.C. (ed.). 1970–1971. *Handbook of Chemistry and Physics,* 51st edition. Chemical Rubber Company, Cleveland, Ohio. 2,063 pp.

Yasuda, H. 1966. Permeability constants. Pp. V13–V24 in *Polymer Handbook* (J. Brandrup and E.H. Immergut, eds.). John Wiley and Sons, New York, New York. 1,050 pp.

Yasuda, H. and V. Stannett. 1975. Permeability coefficients. Pp. III229–III240 in *Polymer Handbook,* 2nd ed. (J. Brandrup and E.H. Immergut, eds.). John Wiley and Sons, New York, New York. 1,363 pp.

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Jones, E.M. and R.D. Owen. 1987. Fluid preservation of specimens. Pp. 51–64 in *Mammal Collection Management* (H.H. Genoways, C. Jones, and O.L. Rossolimo, eds.). Texas Tech University Press, Lubbock, Texas. 219 pp.

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